US 20110252505A1

(19) **United States**

(12) **Patent Application Publication**

Bao et al.

(10) **Pub. No.: US 2011/0252505 A1**

(43) **Pub. Date: Oct. 13, 2011**

(54) **DOWN-REGULATION OF ACC SYNTHASE FOR IMPROVED PLANT PERFORMANCE**

(75) Inventors: **Xiaoming Bao**, Johnston, IA (US); **Nicholas J. Bate**, Raleigh, NC (US); **Jeffrey E. Habben**, Urbandale, IA (US); **Honor Renee Lafitte**, Davis, CA (US); **Kellie Reimann**, Ankeny, IA (US)

(73) Assignee: **PIONEER HI-BRED INTERNATIONAL, INC.**, Johnston, IA (US)

(21) Appl. No.: **12/897,489**

(22) Filed: **Oct. 4, 2010**

Related U.S. Application Data

(60) Provisional application No. 61/248,060, filed on Oct. 2, 2009, provisional application No. 61/290,902, filed on Dec. 30, 2009, provisional application No. 61/332,069, filed on May 6, 2010.

Publication Classification

(51) **Int. Cl.**

A01H 5/00	(2006.01)
A01H 5/10	(2006.01)
C12N 15/82	(2006.01)
C12N 15/113	(2010.01)
C12N 5/10	(2006.01)

(52) **U.S. Cl.** **800/283**; 536/24.1; 435/419; 800/298; 435/412; 435/415; 435/416; 800/278; 435/320.1; 800/320.1; 800/320.3; 800/320.2; 800/320; 800/312; 800/306; 800/322

(57) **ABSTRACT**

Methods for modulating plants using optimized ACC synthase down-regulation constructs are disclosed. Also disclosed are nucleotide sequences, constructs, vectors, and modified plant cells, as well as transgenic plants displaying increased seed and/or biomass yield, improved tolerance to abiotic stress such as drought or high plant density, improved nitrogen utilization efficiency and/or reduction in ethylene production.

FIGURE 1

General Description

DNA SEQ ID NO:3; UBI:ZM-ACS6 RNAi + UBI:MOPAT:PINII Co-integrate.

length: 51280 bp; storage type: Basic; form: Circular

Functional Map

CDS (10 signals)

MO-PAT Start: 7109 End: 7660

SPC Start: 9525 End: 10313 (Complementary) SPECTINOMYCIN RESISTANCE

TET Start: 14622 End: 15272 (Complementary) tetracycline resistance

TET Start: 15378 End: 16028 (Complementary) tetracycline resistance

TRF A Start: 17208 End: 19397 (Complementary)

CTL Start: 24763 End: 31033 (Complementary)

VIR C1 Start: 34264 End: 34958

VIR C2 Start: 34961 End: 35569

VIR G Start: 35680 End: 36483 (Complementary) Agrobacterium virG (region approximated)

VIR B Start: 36615 End: 46051 (Complementary) Agrobacterium virB (region approximated)

Intron (3 signals)

UBI1ZM INTRON1 (PHI) Start: 2196 End: 3208

ADH1 INTRON1 (PHI) Start: 3791 End: 4327 Isolated from B73

UBI1ZM INTRON1 (PHI) Start: 6060 End: 7072

Miscellaneous feature (11 signals)

RB Start: 1 End: 25

ALL STOPS Start: 306 End: 339

A synthetic sequence of stop codons designed to stop all 6 open reading frames.

FRT5 Start: 452 End: 499

ALL STOPS Start: 910 End: 943

ATTB1 Start: 1155 End: 1175

ATTB2 Start: 4931 End: 4951 (Complementary)

FRT12 Start: 4998 End: 5045 FLP recombination target 12

FRT1 Start: 8004 End: 8051

PSB1 Start: 8052 End: 8146

A synthetic sequence designed to facilitate PCR analysis of recombined FRT sites.

FIGURE 1

ALL STOPS Start: 8147 End: 8180

LB Start: 8325 End: 8350 tDNA Left border sequence

Promoter prokaryotic (2 signals)

UBI1ZM PRO Start: 1218 End: 2113 Maize ubiquitin promoter

UBI1ZM PRO Start: 5082 End: 5977 Maize ubiquitin promoter

Rep origin (2 signals)

COLE1 ORI Start: 11588 End: 11857 (Complementary)

ORI V Start: 32041 End: 32751 (Complementary)

Terminator (2 signals)

IN2-1 (B) TERM Start: 505 End: 902 (Complementary)

98bp deletion from 3'-end of the terminator.

PINII TERM Start: 7671 End: 7989; Potato PINII terminator

5'UTR (2 signals)

UBI1ZM 5UTR (PHI) Start: 2114 End: 2195

UBI1ZM 5UTR (PHI) Start: 5978 End: 6059

Misc RNA (2 signals)

ZM-ACS6 (TR3) Start: 3272 End: 3776 (Complementary)

Maize ACC synthase 6 (Aminocyclopropane carboxylate synthase) Truncated fragment for gene silencing.

ZM-ACS6 (TR4) Start: 4332 End: 4874

Maize ACC synthase 6 (Aminocyclopropane carboxylate synthase) Truncated fragment for gene silencing.

Restriction Map

ApaLI: 14 sites G|TGCAC
CACGT|G

N1: 287; N2: 2517; N3: 3704; N4: 4391; N5: 6381; N6: 10036; N7: 10810; N8: 11376

N9: 11874; N10: 13564; N11: 45696; N12: 48291; N13: 48789; N14: 50471

AvaI: 37 sites C|YCGRG
GRGCY|C

N1: 1144; N2: 1909; N3: 3318; N4: 3666; N5: 4429; N6: 4777; N7: 4922; N8: 5072

N9: 5773; N10: 7116; N11: 10391; N12: 15995; N13: 16761; N14: 17741; N15: 20628

N16: 21650; N17: 22422; N18: 24908; N19: 28599; N20: 30815; N21: 31984

N22: 33215; N23: 33223; N24: 33231; N25: 34047; N26: 34545; N27: 35022

N28: 35445;N29: 35491; N30: 36128; N31: 36869; N32: 37358; N33: 37895

N34: 38948; N35: 41282; N36: 43949; N37: 51174

FIGURE 1

BamHI: 11 sites G|GATCC
CCTAG|G

N1: 3230; N2: 4329; N3: 4865; N4: 5066; N5: 7094; N6: 34434; N7: 35775; N8: 37634

N9: 38570; N10: 38770; N11: 43916

ClaI: 18 sites AT|CGAT
TAGC|TA

N1: 2445; N2: 2710; N3: 2935; N4: 6309; N5: 6574; N6: 6799; N7: 33943; N8: 34189

N9: 34622; N10: 36384; N11: 36728; N12: 36855; N13: 41483; N14: 42530; N15: 44527

N16: 46781; N17: 47943; N18: 48057

EcoRI: 11 sites G|AATTC
CTTAA|G

N1: 2609; N2: 3261; N3: 4834; N4: 4877; N5: 6473; N6: 13890; N7: 37680; N8: 40564

N9: 40976; N10: 42287; N11: 43685

HindIII: 8 sites A|AGCTT
TTCGA|A

N1: 649; N2: 1202; N3: 3864; N4: 33254; N5: 44317; N6: 45226; N7: 46347; N8: 47862

NcoI: 2 sites C|CATGG
GGTAC|C

N1: 17026; N2: 17554

PstI: 15 sites CTGCA|G
G|ACGTC

N1: 1218; N2: 3208; N3: 3777; N4: 5082; N5: 7072; N6: 12698; N7: 13142; N8: 33407

N9: 38321; N10: 41155; N11: 42206; N12: 42791; N13: 48045; N14: 49613; N15: 50049

SmaI: 10 sites CCC|GGG
GGG|CCC

N1: 1146; N2: 3320; N3: 3668; N4: 4431; N5: 4779; N6: 4924; N7: 5074; N8: 15997

N9: 16763; N10: 34049

Figure 2. UBI:ZM-ACS6 RNAi Events under Flowering Stress - Environment 1

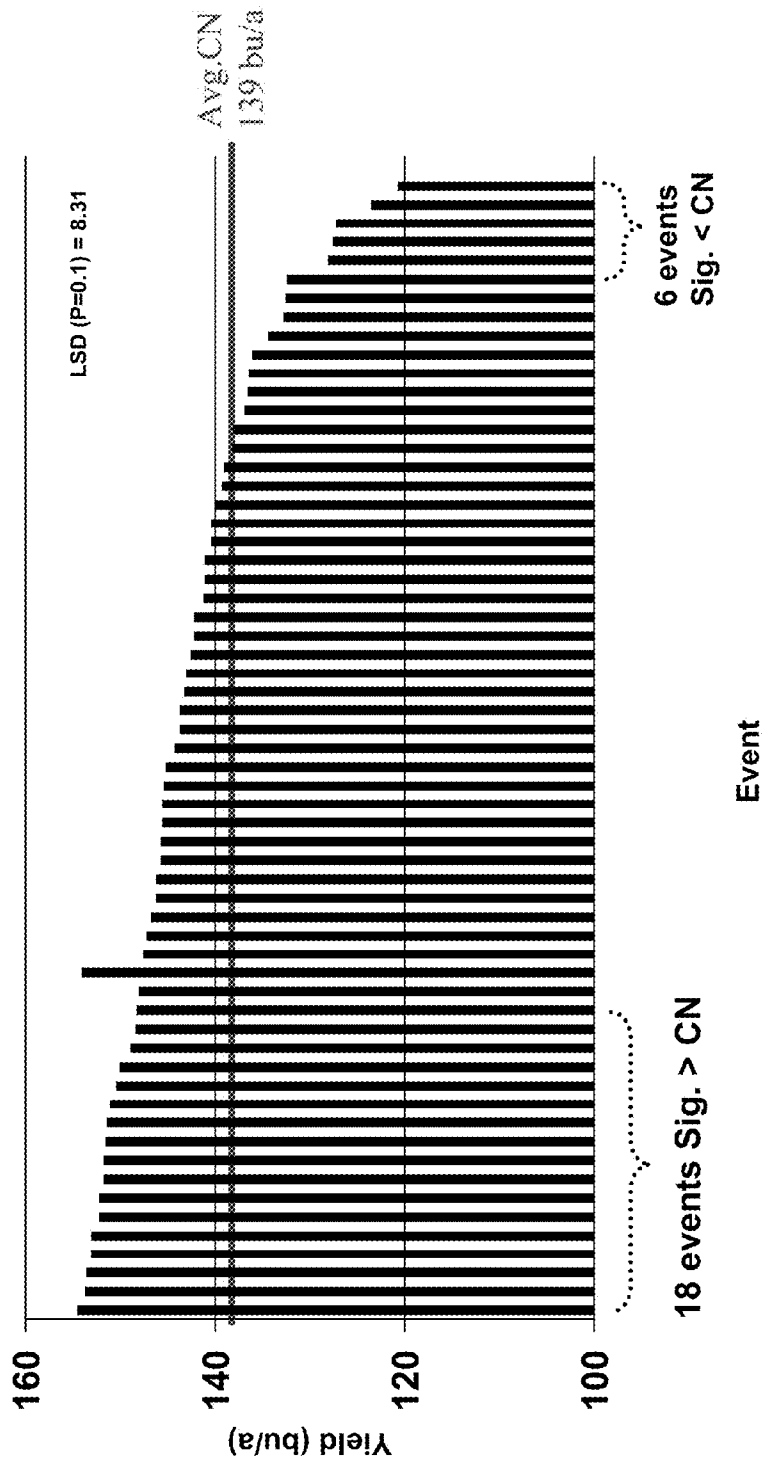


Figure 3. UBI:ZM-ACS6 RNAi Events
under Grain Fill Stress - Environment 2

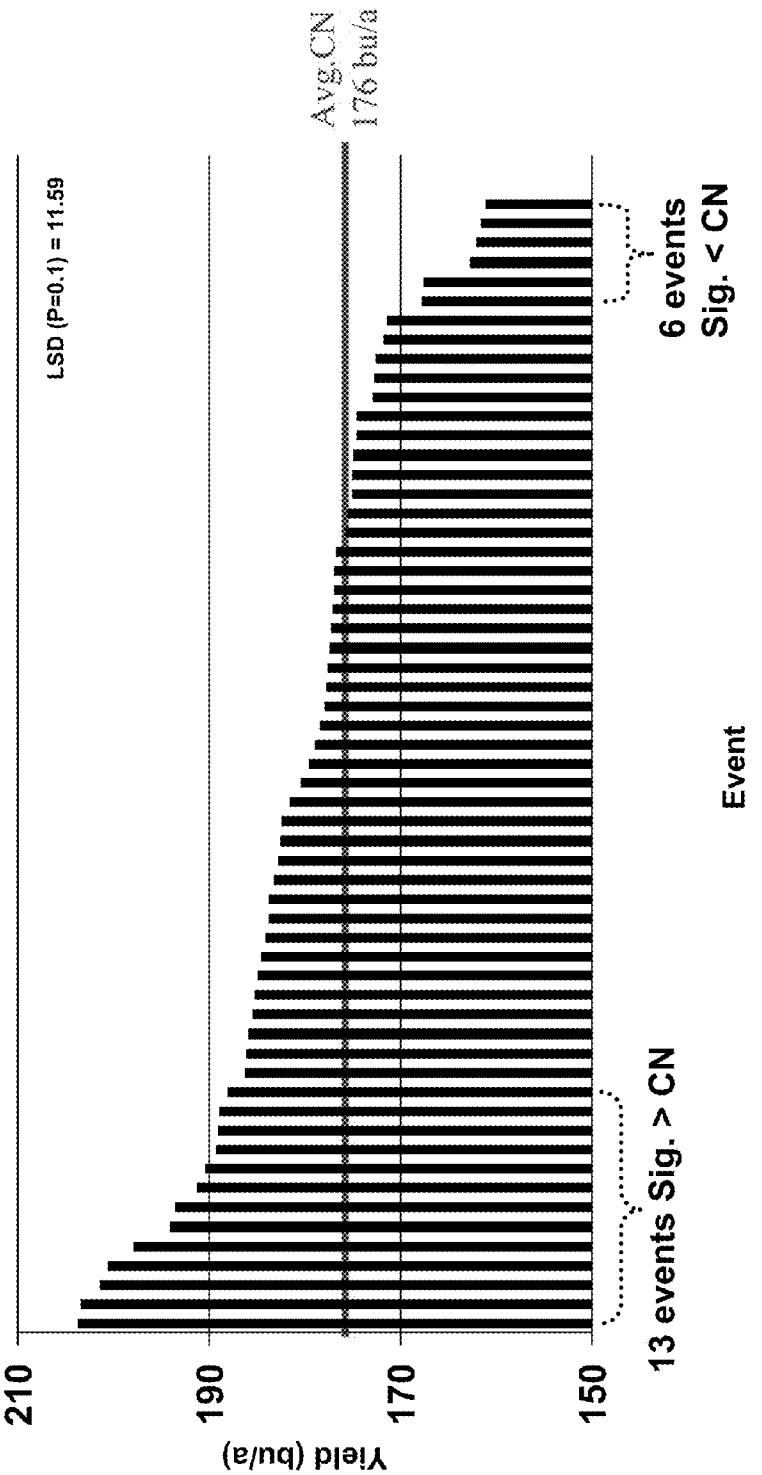


Figure 4. UBI:ZM-ACS6 RNAi Events under Grain Fill Stress - Environment 3

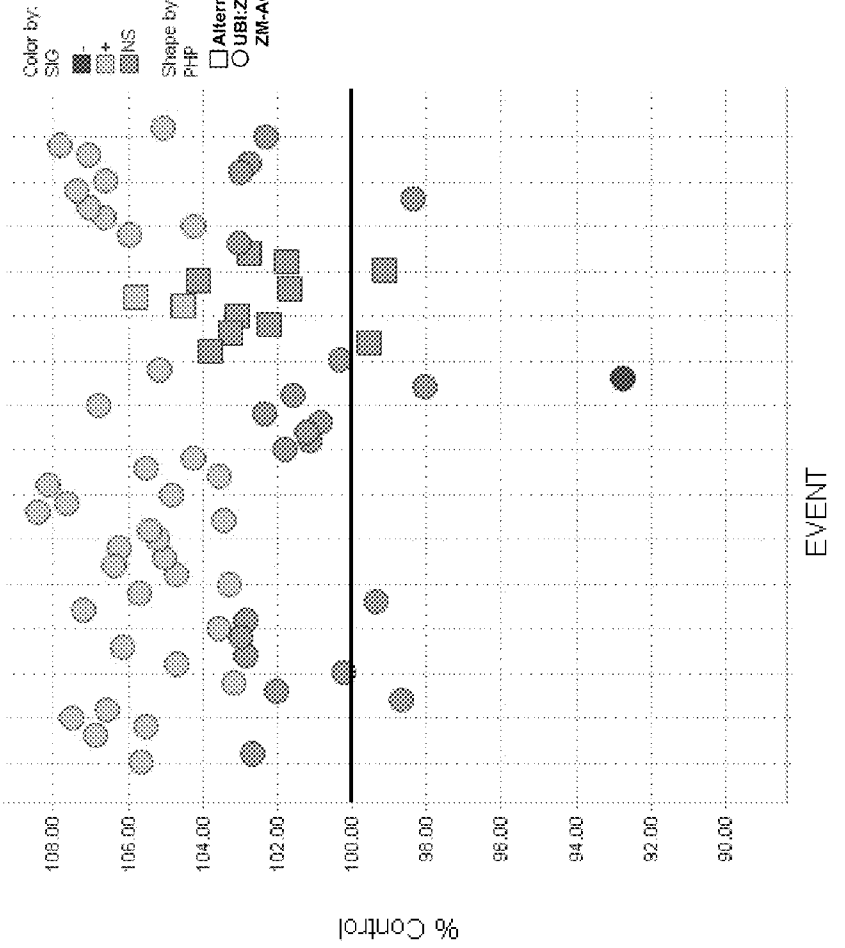
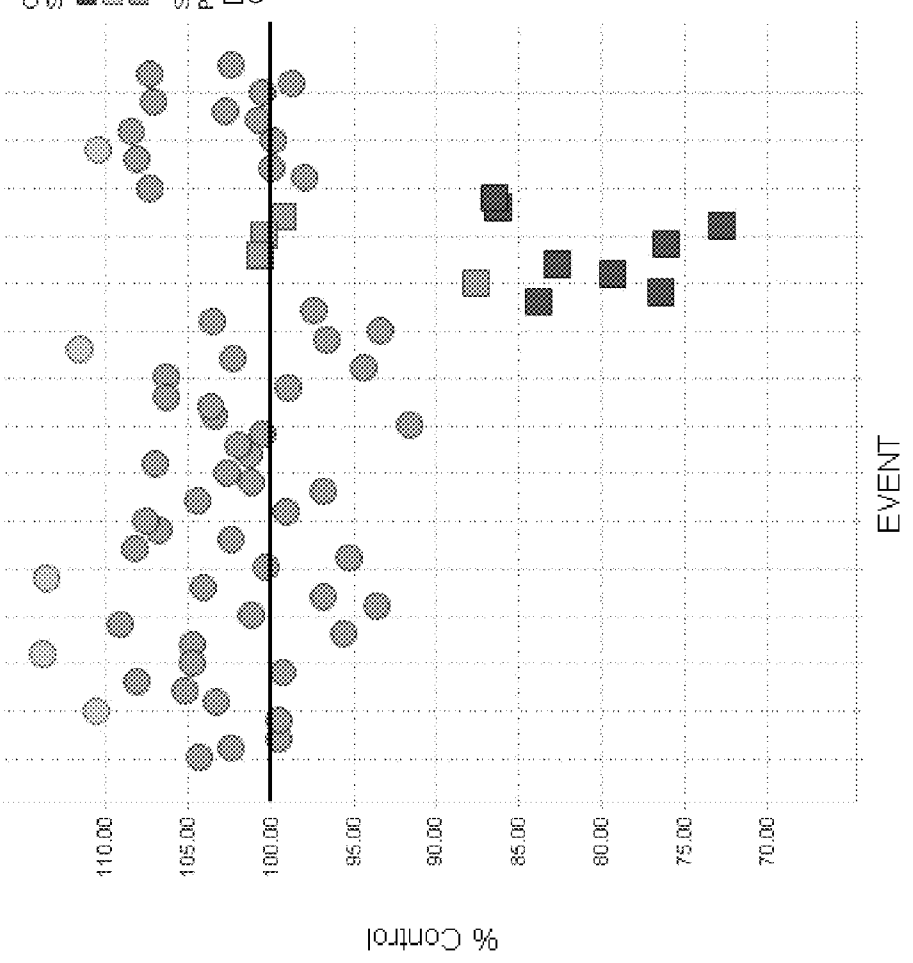


Figure 5. UBI:ZM-ACS6 RNAi Events under Rain-Fed Conditions - Enviroment 4



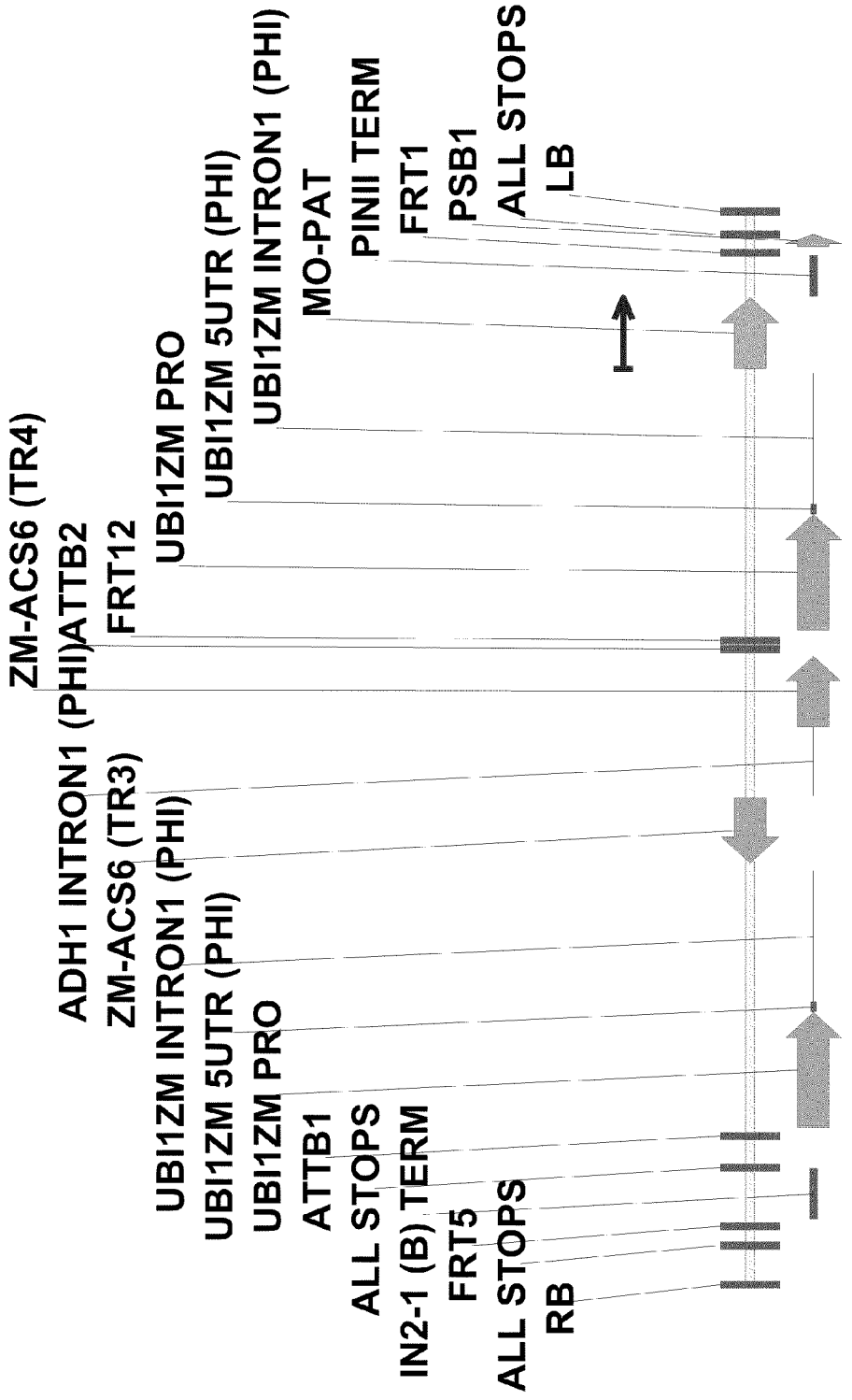


Figure 6. Expression cassette; 8350 bp.

```
BestFit Results

BESTFIT of: maize_ACS6_TR4 from: 1  to: 543

        to: rice_ACS6_cds  from: 1  to: 1464

 Symbol comparison table: /app/gcg/share/matrix/swgapdna.cmp
 CompCheck: 2335

         Gap Weight:     50      Average Match: 10.000
      Length Weight:      3   Average Mismatch: -9.000

            Quality:   3373             Length:    476
              Ratio:  7.086              Gaps:      0
 Percent Similarity: 84.664   Percent Identity: 84.664

        Match display thresholds for the alignment(s):
                    | = IDENTITY
                    : =   5
                    . =   1

maize_ACS6_TR4 x rice_ACS6_cds September 24, 2010 00:41  ..

                .         .         .         .         .
      19 ggacgacggcgtcgtcggcgttgggccgctgtcggaccgcgtgcacgtgg 68
         || ||   ||| || |||||  |      |||| ||||||||||||||| |
     792 ggccgggcgcgacggcggcggcgctgacgtgtccgaccgcgtgcacgtcg 841
                .         .         .         .         .
      69 tgtacagcctgtccaaggacctgggcctcccggggttccgcgtgggcgcc 118
         ||||||||||||||||||||| ||||||||||||||||||||| ||||||
     842 tgtacagcctgtccaaggacctcggcctcccggggttccgcgtcggcgcc 891
                .         .         .         .         .
     119 atctactcgtccaacgccggcgtggtctccgcggccaccaagatgtccag 168
         ||||||||  |||||||||| ||| || |||||||| ||||||||||| ||
     892 atctactccgccaacgccgccgtcgtgtccgcggcgaccaagatgtccag 941
                .         .         .         .         .
     169 cttcggcctggtgtcgtcccagacgcagcacctcctggcgtcgctcctgg 218
         ||||||||| |||||||||||||||| ||||||| ||| |||| || |
     942 cttcggcctcgtgtcgtcccagacgcagtacctcctcgcggcgctgctcg 991
                .         .         .         .         .
     219 gcgacagggacttcacgcggaggtacatcgcggagaacacgcggccggatc 268
         ||||||||||||||| ||||| ||| |||||||||||| ||||||||||
     992 gcgacagggacttcacccggagctacgtcgcggagaacaagcggccggatc 1041
                .         .         .         .         .
     269 agggagcggcgcgagcagctggcggagggcctggcggccgtgggcatcga 318
         | |||||||| ||| ||||| | ||| || ||   ||   | ||||| |
    1042 aaggagcggcacgaccagctcgtggacgggctcagggagatcggcattgg 1091
                .         .         .         .         .
     319 gtgcctggagagcaacgcgggctcttctgctgggtcaacatgcggcgcc 368
         |||||||   |||||||| || ||||||||||||||  ||||| | | ||
    1092 gtgcctgcccagcaacgccggcctcttctgctgggtggacatgagccacc 1141
                .         .         .         .         .
     369 tgatgcggagccggtcgttcgagggcgagatggagctgtggaagaggctg 418
         ||||||||||||||||||||  |||||||||||||| ||||||| ||||
    1142 tgatgcggagccggtcgttcgccggcgagatggagctctggaagaaggctg 1191
                .         .         .         .         .
     419 gtcttcgaggtggggctcaacatctccccgggctcctcctgccactgccg 468
         || |||||||| || |||||||||||||| || || || ||||||||||
    1192 gtgttcgaggtcggcctcaacatctcccccgggtcgtcgtgccactgccg 1241
                .         .
     469 ggagcccggctggttccgcgtctgct 494
          |||||||||||||||||||||||||
    1242 cgagcccggctggttccgcgtctgct 1267
```

FIGURE 7

```
BESTFIT of: ZmACS6 cDNA  check: 3296  from: 1  to: 1446


to: rice_ACS6_cds  check: 9272  from: 1  to: 1464

 REFORMAT of: rice_ACS6_cds  check: 9272  from: 1  to: 1464
 (No documentation)

 Symbol comparison table: /app/gcg/share/matrix/swgapdna.cmp
 CompCheck: 2335

        Gap Weight:     50      Average Match: 10.000
     Length Weight:      3   Average Mismatch: -9.000

           Quality:  10153             Length:   1474
             Ratio:  7.031               Gaps:      7
 Percent Similarity: 87.015   Percent Identity: 87.015

        Match display thresholds for the alignment(s):
                    | = IDENTITY
                    : =   5
                    . =   1

ZmACS6 cDNA x rice_ACS6_cds

               .         .         .         .         .
      2 TGATCGCCGACGAGAAGCCGCAGCCGCAGCTGCTGTCCAAGAAGGCCGCC 51
        || ||||||| ||||      ||||||||||||||||||||||||||||
     14 tggtcgccgaggaga......agccgcagctgctgtccaagaaggccggg 57
               .         .         .         .         .
     52 TGCAACAGCCACGGCCAGGACTCGTCCTACTTCCTGGGGTGGGAGGAGTA 101
        |||||||||||||||||||||||||||||||||||||||||| |||||||
     58 tgcaacagccacggccaggactcgtcctacttcctggggtggcaggagta 107
               .         .         .         .         .
    102 TGAGAAAAACCCATACGACCCCGTCGCCAACCCCGGCGGCATCATCCAGA 151
         |||||||||| | |||||||||| |||||||   |||||||||||||
    108 cgagaaaaacccgttcgaccccgtctccaaccccttccggcatcatccaga 157
               .         .         .         .         .
    152 TGGGCCTCGCCGAGAACCAGCTGTCCTTCGACCTGCTGGAGGCGTGGCTG 201
        |||||||||||||||||||||||| ||| |||||||| |||| |||||||
    158 tgggcctcgccgagaaccagctgtcgttcgacctgcttgaggagtggctg 207
               .         .         .         .         .
    202 GAGGCCAACCCGGACGCGCTCGGCCTCCGCC......GGGGAGGCGCCTC 245
        |||   |||||  ||||||||||||||| |      | || ||||||||
    208 gagaagaaccccccacgcgctcggcctccggcgagagggcggcggcgcctc 257
               .         .         .         .         .
    246 TGTATTCCGCGAGCTCGCGCTCTTCCAGGACTACCACGGCATGCCGGCCT 295
         || ||||||||||||||| |||||||||||||||||| | ||||| |
    258 cgtcttccgcgagctcgcgctgttccaggactaccacggcctcccggctt 307
               .         .         .         .         .
    296 TCAAGAATGCATTGGCGAGGTTCATGTCGGAGCAACGTGGGTACCGGGTG 345
        |||| |||||||||||| ||||||||||||||||  | ||||||  ||||
    308 tcaaaaatgcattggcgcggttcatgtcggagcagagagggtacaaggtg 357
               .         .         .         .         .
    346 ACCTTCGACCCCAGCAACATCGTGCTCACCGCCGGAGCCACCTCGGCCAA 395
           |||||||||||||||||||||||||||||| |||||||||| ||
    358 gtgttcgaccccagcaacatcgtgctcaccgccggcgccacctcggctaa 407
               .         .         .         .         .
    396 CGAGGCCCTCATGTTCTGCCTCGCCGACCACGGAGACGCCTTTCTCATCC 445
        |||||| |||||||||||||||||||||||| ||||||| |||||||
    408 cgaggcgctcatgttctgcctcgccgaccacggcgacgccttcctcatcc 457
               .         .         .         .         .
    446 CCACGCCATACTACCCAGGGTTCGACCGTGACCTCAAGTGGCGCACCGGC 495
        |||| |||||||||||||||||||| ||||||||||||||||||||||
    458 ccaccccatactacccagggttcgaccgcgacctcaagtggcgcaccggc 507
               .         .         .         .         .
    496 GCGGAGATCGTCCCCGTGCACTGCACGAGCGGCAACGGCTTCCGGCTGAC 545
        |||||||||| |||||||||| ||||||  ||||| |||||| ||||
    508 gcggagatcgtacccgtgcactgcgcgagcgcgaacgggttccgggtgac 557
```

FIGURE 8A

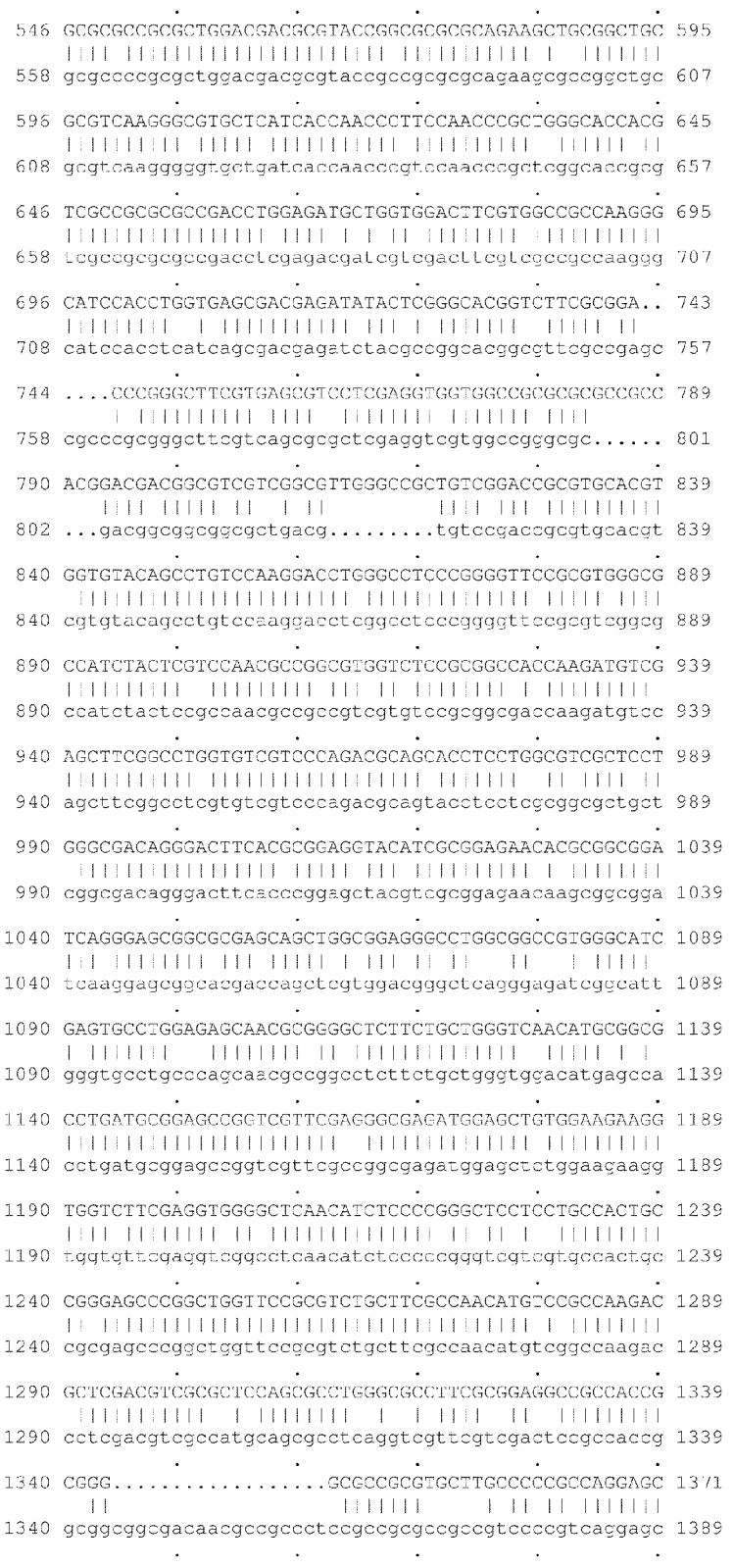


FIGURE 8B

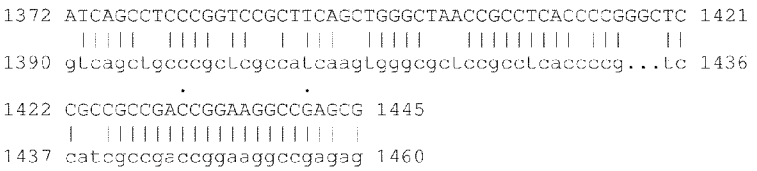

1372 ATCAGCCTCCCGGTCCGCTTCAGCTGGGCTAACCGCCTCACCCCGGGCTC 1421
1390 gtcagctgcccgctcgccatcaagtgggcgctccgcctcaccccg...tc 1436
1422 CGCCGCCGACCGGAAGGCCGAGCG 1445
1437 catcgccgaccggaaggccgagag 1460

FIGURE 8C

Figure 9.

	Rain-fed			Drought stress	
Event	**% of WT**	**% of BN**		**% of WT**	**% of BN**
Background 1, Event 4	100	102		107	111
Background 1, Event 6	99	101		105	109
Background 1, Event 7	101	103		106	110
Background 1, Event 1	98	100		104	108
Background 1, Event 3	100	102		102	106
Background 1, Event 8	100	102		101	105
Background 1, Event 2	98	100		111	116
Background 1, Event 9	100	102		99	103
Background 1, Event 10	101	103		102	106

Background	Event	Across Testers & Locations
1	1	64
1	2	70
1	3	72
1	4	69
1	5	66
1	6	68
1	7	70
1	8	71
1	9	70
1	10	68
1	11	69
1	12	69
	BN	**63**
	WT	**63**

Grain yield (bushels/acre)of transgenic, bulk null (BN) and nontransformed (WT) maize hybrids in Season 3

Significantly greater than BN and WT

FIGURE 10

Event	Background	Across Testers & Locations
1	1	110
2	1	110
3	1	111
4	1	110
5	1	110
6	1	110
7	1	110
8	1	110
9	1	110
10	1	111
11	1	110
12	1	110
BN		**108**
WT		**107**

Plant height (inches) of transgenic, bulk null (BN) and nontransformed (WT) maize hybrids in Season 3

Significantly different from BN and WT

FIGURE 11

Background	Event	Yield – Across Testers & Locations	Plant Height – Across Testers & Locations
3	1	111	120
3	2	109	120
3	3	120	120
3	4	117	120
3	5	115	120
3	6	122	120
3	7	124	120
3	8	116	120
3	9	121	120
3	10	112	120
3	11	117	120
3	12	118	120
3	13	113	120
2	1	118	120
2	2	118	120
2	3	117	120
2	4	122	120
2	5	118	120
2	6	119	120
2	7	119	121
2	8	119	120
2	9	119	119
2	10	119	120
2	11	115	120
BN		**116**	**117**
WT		**119**	**118**

FIGURE 12

Significantly different from BN only

Significantly different from WT only

Significantly different from both BN and WT

Event	Background 3
1	186
2	185
3	183
4	182
5	181
6	181
7	181
8	181
9	180
10	180
11	180
12	179
13	176
WT	**172**

Event	Background 2
1	176
2	175
3	174
4	174
5	173
6	174
7	172
8	172
9	171
10	172
11	171
BN	**167**

Seed yield (bu/acre) of transgenic, bulk null (BN) and nontransformed (WT) maize hybrids across three water treatments and four testers in Season 4.

Significantly greater than control

FIGURE 13

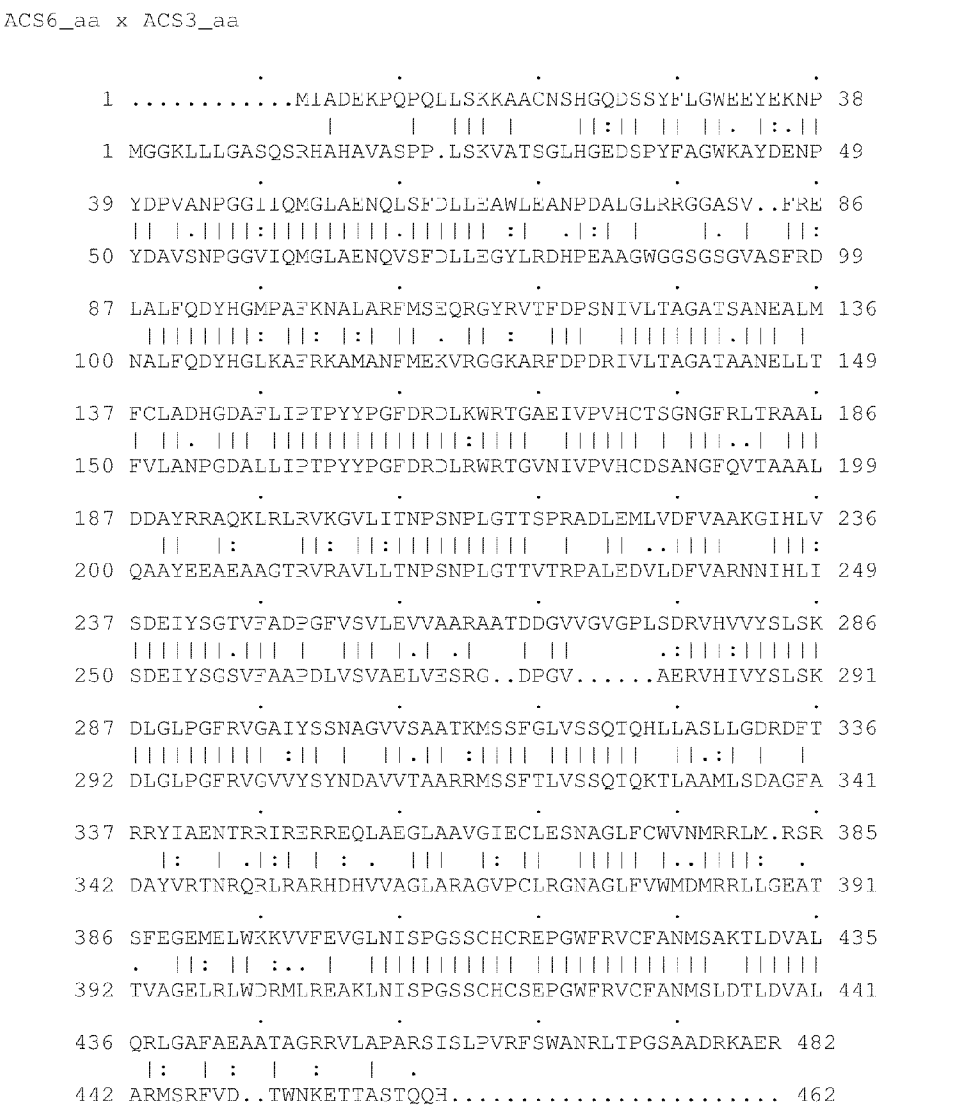

ACS6_aa x ACS3_aa
1 .....MIADEKPQPQLLSKKAACNSHGQDSSYFLGWEEYEKNP 38
1 MGGKLLLGASQSRHAHAVASPP.LSKVATSGLHGEDSPYFAGWKAYDENP 49
39 YDPVANPGGIIQMGLAENQLSFDLLEAWLEANPDALGLRRGGASV..FRE 86
50 YDAVSNPGGVIQMGLAENQVSFDLLEGYLRDHPEAAGWGGSGSGVASFRD 99
87 LALFQDYHGMPAFKNALARFMSEQRGYRVTFDPSNIVLTAGATSANEALM 136
100 NALFQDYHGLKAFRKAMANFMEKVRGGKARFDPDRIVLTAGATAANELLT 149
137 FCLADHGDAFLIPTPYYPGFDRDLKWRTGAEIVPVHCTSGNGFRLTRAAL 186
150 FVLANPGDALLIPTPYYPGFDRDLRWRTGVNIVPVHCDSANGFQVTAAAL 199
187 DDAYRRAQKLRLRVKGVLITNPSNPLGTTSPRADLEMLVDFVAAKGIHLV 236
200 QAAYEEAEAAGTRVRAVLLTNPSNPLGTTVTRPALEDVLDFVARNNIHLI 249
237 SDEIYSGTVFADPGFVSVLEVVAARAATDDGVVGVGPLSDRVHVVYSLSK 286
250 SDEIYSGSVFAAPDLVSVAELVESRG..DPGV......AERVHIVYSLSK 291
287 DLGLPGFRVGAIYSSNAGVVSAATKMSSFGLVSSQTQHLLASLLGDRDFT 336
292 DLGLPGFRVGVVYSYNDAVVTAARRMSSFTLVSSQTQKTLAAMLSDAGFA 341
337 RRYIAENTRRIRERREQLAEGLAAVGIECLESNAGLFCWVNMRRLM.RSR 385
342 DAYVRTNRQRLRARHDHVVAGLARAGVPCLRGNAGLFVWMDMRRLLGEAT 391
386 SFEGEMELWKKVVFEVGLNISPGSSCHCREPGWFRVCFANMSAKTLDVAL 435
392 TVAGELRLWDRMLREAKLNISPGSSCHCSEPGWFRVCFANMSLDTLDVAL 441
436 QRLGAFAEAATAGRRVLAPARSISLPVRFSWANRLTPGSAADRKAER 482
442 ARMSRFVD..TWNKETTASTQQH..... 462
Symbol comparison table: /app/gcg/share/matrix/blosum62.cmp
CompCheck: 1102
BLOSUM62 amino acid substitution matrix.
Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid
substitution matrices from protein blocks. PNAS USA 89: 10915-10919.
Gap Weight: 8 Average Match: 2.778
Length Weight: 2 Average Mismatch: -2.248
Quality: 1318 Length: 497
Ratio: 2.853 Gaps: 6
Percent Similarity: 65.996 Percent Identity: 59.284
Match display thresholds for the alignment(s):
| = IDENTITY
: = 2
. = 1

FIGURE 14

FIGURE 15

ZmACS3_cDNA x ZmACS6_cDNA

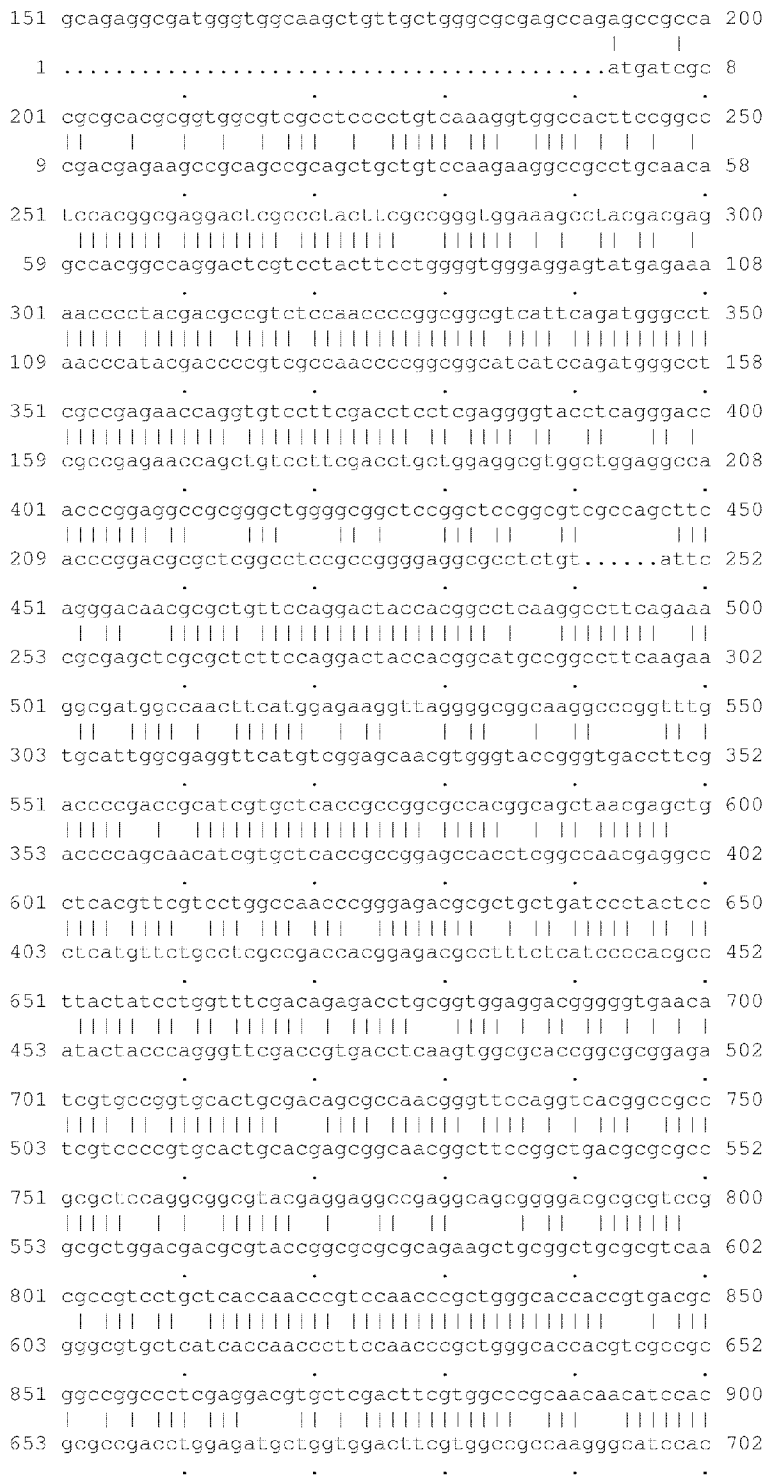


FIGURE 15

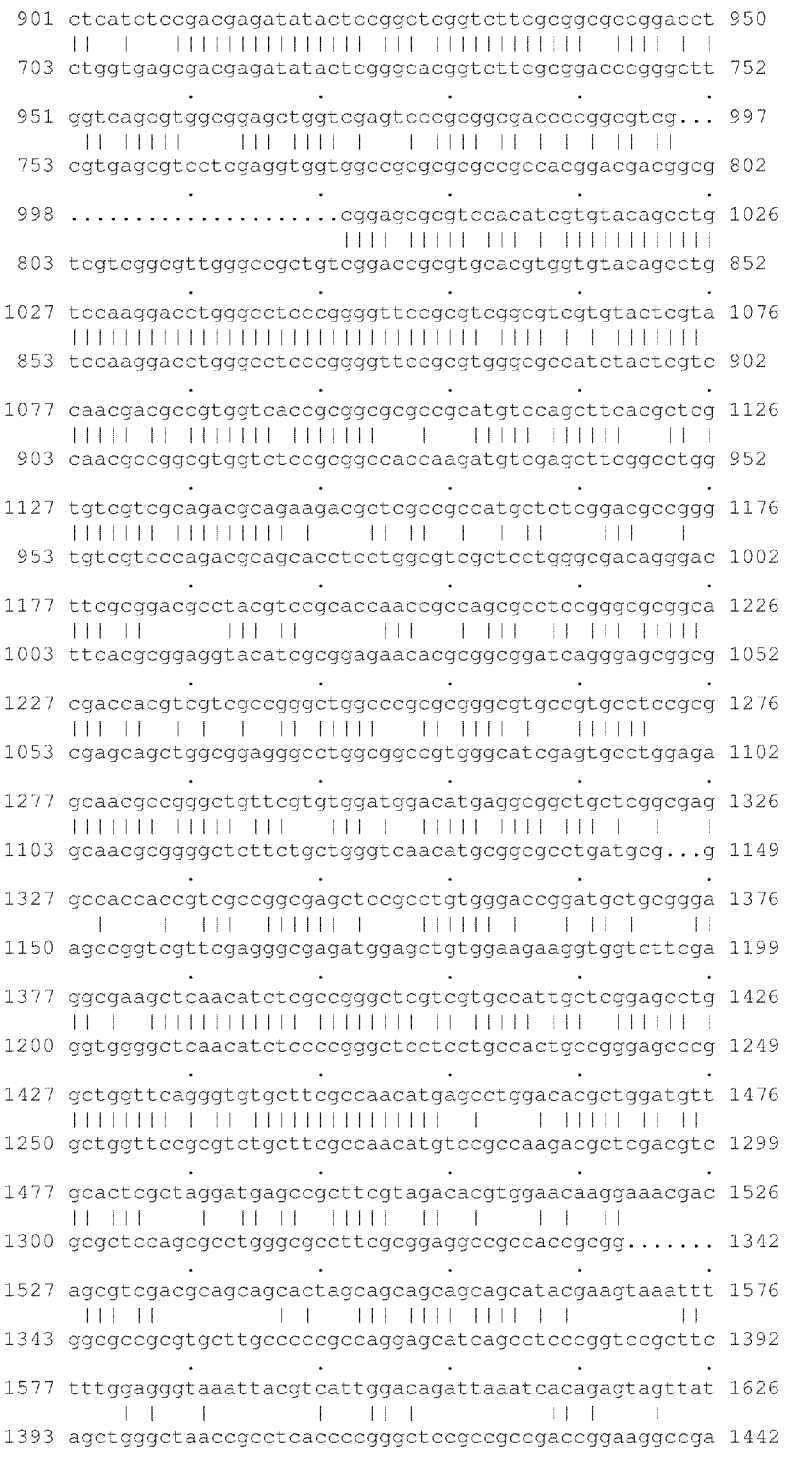


FIGURE 15

```
    1627 acaggggattcttttatggtttttcgattgatggtaacatcgattttgt 1676
           | |
    1443 gcgg........................................... 1446
                                  .
                                  .


Symbol comparison table: /app/gcg/share/matrix/nwsgapdna.cmp
 CompCheck: 8760

        Gap Weight:     50      Average Match: 10.000
     Length Weight:      3   Average Mismatch:  0.000

           Quality:   9040             Length:   1783
             Ratio:  6.252               Gaps:      4
Percent Similarity: 65.823   Percent Identity: 65.823

       Match display thresholds for the alignment(s):
                   | = IDENTITY
                   : =   5
                   . =   1                 .
```

```
hpRNA    (..8) ----------------CGCGCCGCCACGGACGACGGCGTCGTCGGCGTTGGGCCGCTGTCG
ZmACS3   (940) GCGCCGGACCTGGTCAGCGTGGCGGAGCTGGTCGAGTCCCGCGGCGACCCCGGCGTCGCG   (999)

hpRNA     (53) GACCGCGTGCACGTGGTGTACAGCCTGTCCAAGGACCTGGGCCTCCCGGGGTTCCGCGTG
ZmACS3  (1000) GAGCGCGTCCACATCGTGTACAGCCTGTCCAAGGACCTGGGCCTCCCGGGGTTCCGCGTC   (1059)

hpRNA    (113) GGCGCCATCTACTCGTCCAACGCCGGCGTGGTCTCCGCGGCCACCAAGATGTCGAGCTTC
ZmACS3  (1060) GGCGTCGTGTACTCGTACAACGACGCCGTGGTCACCGCGGCGCGCCGCATGTCCAGCTTC   (1119)

hpRNA    (173) GGCCTGGTGTCGTCCCAGACGCAGCACCTCCTGGCGTCGCTCCTGGGCGACAGGGACTTC
ZmACS3  (1120) ACGCTCGTGTCGTCGCAGACGCAGAAGACGCTCGCCGCCATGCTCTCGGACGCCGGGTTC   (1179)

hpRNA    (233) ACGCGGAGGTACATCGCGGAGAACACGCGGCGGATCAGGGAGCGGCGCGAGCAGCTGGCG
ZmACS3  (1180) GCGGACGCCTACGTCCGCACCAACCGCCAGCGCCTCCGGGCGCGGCACGACCACGTCGTC   (1239)

hpRNA    (293) GAGGGCCTGGCGGCCGTGGGCATCGAGTGCCTGGAGAGCAACGCGGGGCTCTTCTGCTGG
ZmACS3  (1240) GCCGGGCTGGCCCGCGCGGGCGTGCCGTGCCTCCGCGGCAACGCCGGGCTGTTCGTGTGG   (1299)

hpRNA    (353) GTCAACATGCGGCGCCTGAT--GCGGAGCCGGT-CGTTCGAGGGCGAGATGGAGCTGTGG
ZmACS3  (1300) ATGGACATGAGGCGGCTGCTCGGCGAGGCCACCACCGTCGCCGGCGAGCTCCGCCTGTGG   (1359)

hpRNA    (410) AAGAGGGTGGTCTTCGAGGTGGGGCTCAACATCTCCCCGGGCTCCTCCTGCCACTGCCGG
ZmACS3  (1360) GACCGGATGCTGCGGGAGGCGAAGCTCAACATCTCGCCGGGCTCGTCGTGCCATTGCTCG   (1419)

hpRNA    (470) GAGCCCGGCTGGTTCCGCGTCTGCT----------------------------------
ZmACS3  (1420) GAGCCTGGCTGGTTCAGGGTGTGCTTCGCCAACATGAGCCTGGACACGCTGGATGTTGCA
```

Figure 16. Alignment of ACS3 (SEQ ID NO: 10) and portion of hpRNA construct (SEQ ID NO: 2) .

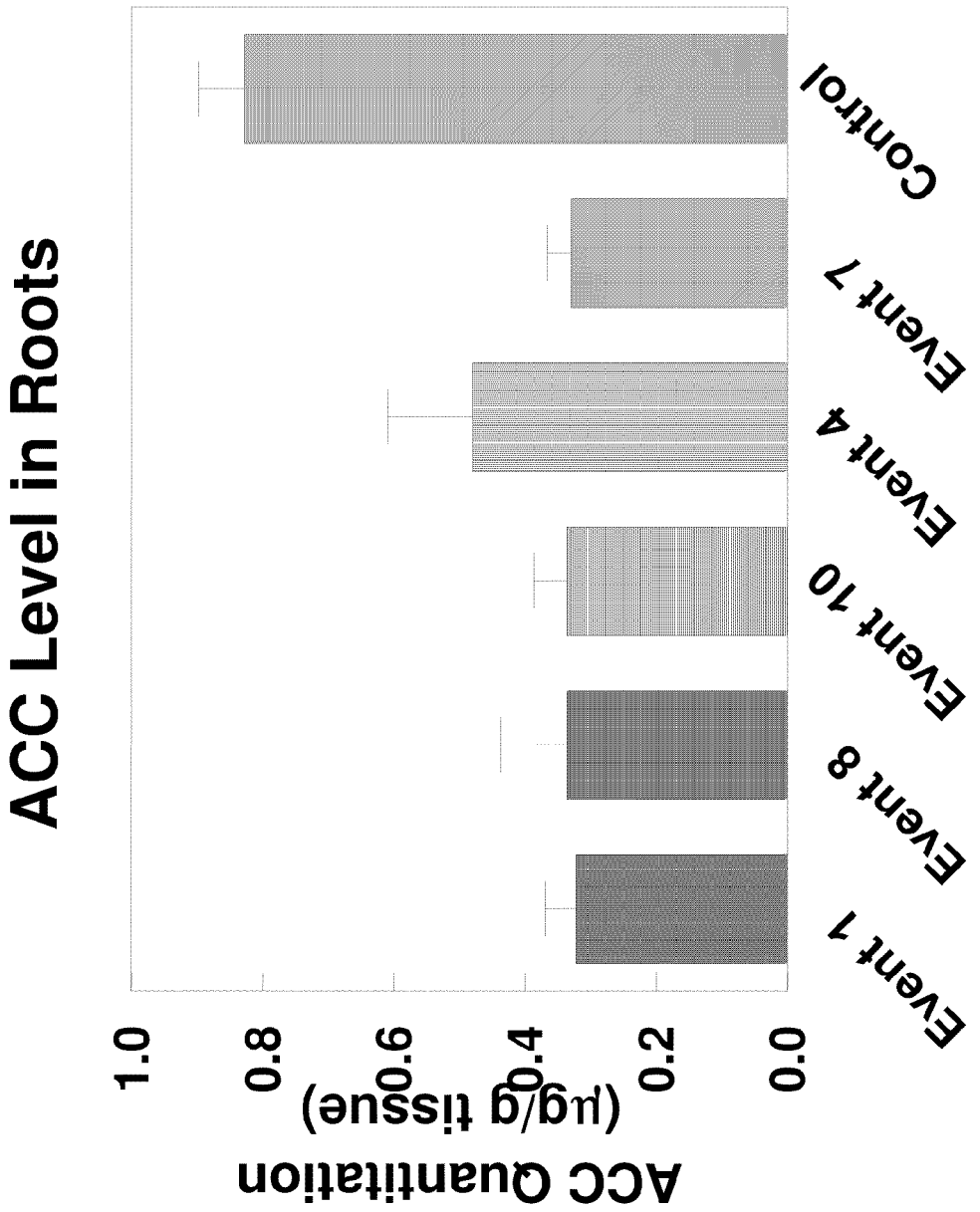

ACC Level in Roots
ACC Quantitation
(µg/g tissue)
1.0
0.8
0.6
0.4
0.2
0.0
Control
Event 7
Event 4
Event 10
Event 8
Event 1

FIGURE 17

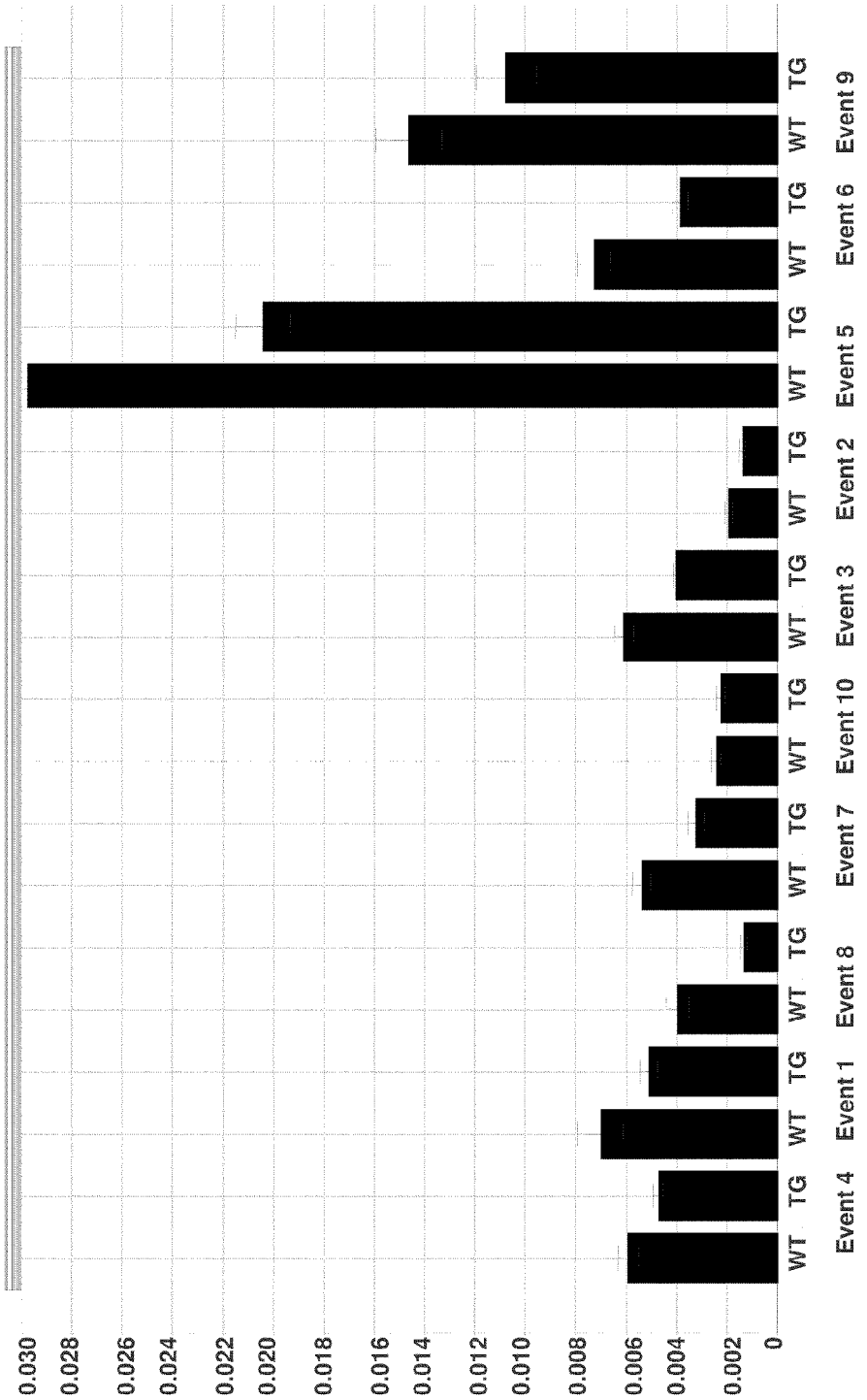

0.030
0.028
0.026
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0
WT TG WT TG WT TG WT TG WT TG WT TG WT TG WT TG WT TG
Event 4 Event 1 Event 8 Event 7 Event 10 Event 3 Event 2 Event 5 Event 6 Event 9

FIGURE 18

DOWN-REGULATION OF ACC SYNTHASE FOR IMPROVED PLANT PERFORMANCE

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application Ser. No. 61/248,060, filed Oct. 2, 2009 and to U.S. Provisional Patent Application Ser. No. 61/290,902, filed Dec. 30, 2009 and to U.S. Provisional Patent Application Ser. No. 61/332,069, filed May 6, 2010, all of which are hereby incorporated by reference herein.

FIELD OF THE INVENTION

[0002] This invention relates generally to the field of molecular biology and the modulation of expression or activity of genes and proteins affecting yield, abiotic stress tolerance and nitrogen utilization efficiency in plants.

BACKGROUND OF THE INVENTION

[0003] Ethylene (C_2H_4) is a gaseous plant hormone. It has a spectrum of effects that can be tissue-specific and/or species-specific. For example, physiological effects include, but are not limited to, promotion of fruit ripening, abscission of leaves and fruit of dicotyledonous species, flower senescence, stem extension of aquatic plants, gas space (aerenchyma) development in roots, leaf epinastic curvature, stem and shoot swelling (often in association with stunting), femaleness in cucurbits, fruit growth in certain species, apical hook closure in etiolated shoots, root hair formation, flowering in the Bromeliaceae, and diageotropism of etiolated shoots. Ethylene is released naturally by ripening fruit and is also produced by most plant tissues, e.g., in response to stress (e.g., density, pathogen attack) and in maturing and senescing organs.

[0004] Ethylene is generated from methionine by a biosynthetic pathway involving the conversion of S-adenosyl-L-methionine (SAM or Ado Met) to the cyclic amino acid 1-aminocyclopropane-1-carboxylic acid (ACC) which is facilitated by ACC synthase. Sulphur is conserved in the process by recycling 5'-methylthioadenosine.

[0005] ACC synthase is an aminotransferase which catalyzes the rate-limiting step in the formation of ethylene by converting S-adenosylmethionine to ACC. Typically, the enzyme requires pyridoxal phosphate as a cofactor. Features of the invention include ACC synthase sequences and subsequences.

[0006] Ethylene is then produced from the oxidation of ACC through the action of ACC oxidase (also known as the ethylene forming enzyme). The ACC oxidase enzyme is stereospecific and uses cofactors, e.g., Fe^{+2}, O_2, ascorbate, etc. Activity of ACC oxidase can be inhibited by anoxia and cobalt ions. Finally, ethylene can be metabolized by oxidation to CO_2 or to ethylene oxide and ethylene glycol.

[0007] The maize ACC synthase (ACS) gene family includes three members: ACS2, ACS6 and ACS7. The identification and analysis of *Arabidopsis* and tomato ACC synthase mutants deficient in ethylene biosynthesis have helped to establish the important role that ethylene plays in plant growth and development. ACC synthase, the first committed enzyme in the ethylene biosynthetic pathway, plays critical regulatory roles throughout cereal development as well as a key role in regulating responses to environmental stress.

[0008] There is a continuing need for modulation of the ethylene production pathway in plants for manipulating plant development or stress responses. This invention relates to the creation of novel ACC synthase downregulation polynucleotide constructs to modulate yield of seed and/or biomass, abiotic stress tolerance, including density tolerance, drought tolerance, nitrogen utilization efficiency and/or ethylene production in plants, including novel polynucleotide sequences, expression cassettes, constructs, vectors, plant cells and resultant plants. These and other features of the invention will become apparent upon review of the following.

SUMMARY OF THE INVENTION

[0009] This invention provides methods and compositions for modulating yield, drought tolerance and/or nitrogen utilization efficiency in plants as well as modulating (e.g., reducing) ethylene production in plants. This invention relates to compositions and methods for down-regulating the level and/or activity of ACC synthase in plants, exemplified by, e.g., SEQ ID NO: 1 and/or SEQ ID NO: 2, including the development of specific RNAi constructs (see, SEQ ID NO: 3) for creation of plants with improved yield and/or improved abiotic stress tolerance, which may include improved drought tolerance, improved density tolerance, and/or improved NUE (nitrogen utilization efficiency). NUE includes both improved yield in low nitrogen conditions and more efficient nitrogen utilization in normal conditions

[0010] Therefore, in one aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide sequence for use in a down-regulation construct, such as an RNAi vector which modulates ACS expression. One embodiment of the invention is an isolated polynucleotide comprising a nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2 which may optimize interaction with endogenous RNA sequences.

[0011] In another aspect, the present invention relates to recombinant down-regulation constructs comprising the polynucleotides as described (see, SEQ ID NO: 3). The down-regulation constructs generally comprise the polynucleotides of SEQ ID NO: 1 and/or SEQ ID NO: 2 and a promoter operably linked to the same. Additionally, the constructs include several features which result in effective down-regulation of ACS through RNAi embodiments or facilitate modulation of ACS expression. One such feature is the inclusion of one or more FLP/FRT sites. Other features include specific elimination of extraneous open reading frames in the hairpin structure, elimination of an open reading frame from the intron of the ubiquitin promoter, alteration of the hairpin to include an Adhl intron and reconfiguration of the construct so that the hairpin cassette and the herbicide-tolerance marker are in tandem orientation. The invention also relates to a vector containing the recombinant expression cassette. Further, the vector containing the recombinant expression cassette can facilitate the transcription of the nucleic acid in a host cell. The present invention also relates to the host cells able to transcribe a polynucleotide.

[0012] In certain embodiments, the present invention is directed to a transgenic plant or plant cell containing a polynucleotide comprising a down-regulation construct. In certain embodiments, a plant cell of the invention is from a dicot or monocot. Preferred plants containing the polynucleotides include, but are not limited to, maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, tomato and millet. In certain embodiments, the transgenic plant is a maize plant or plant cell. A transgenic seed comprising a transgenic down-regulation construct as described herein is an embodiment. In one embodiment, the plant cell is in a

hybrid plant comprising a drought tolerance phenotype and/or a nitrogen utilization efficiency phenotype and/or an improved yield phenotype. In another embodiment, the plant cell is in a plant comprising a sterility phenotype, e.g., a male sterility phenotype. Plants may comprise a combination of such phenotypes. A plant regenerated from a plant cell of the invention is also a feature of the invention.

[0013] Certain embodiments have improved drought tolerance as compared to a control plant. The improved drought tolerance of a plant of the invention may reflect physiological aspects such as, but not limited to, (a) a reduction in the production of at least one ACC-synthase-encoding mRNA; (b) a reduction in the production of an ACC synthase; (c) a reduction in the production of ACC; (d) a reduction in the production of ethylene; (e) an increase in plant height or (f) any combination of (a)-(e), compared to a corresponding control plant. Plants exhibiting improved drought tolerance may also exhibit one or more additional abiotic stress tolerance phenotypes, such as improved nitrogen utilization efficiency and increased density tolerance.

[0014] The invention also provides methods for inhibiting ethylene production in a plant and plants produced by such methods. For example, a method of inhibiting ethylene production comprises inhibiting the expression of one or more ACC synthase genes in the plant, wherein the one or more ACC synthase genes encode one or more ACC synthases. Multiple methods and/or multiple constructs may be used to downregulate a single ACC synthase polynucleotide or polypeptide. Multiple ACC synthase polynucleotides or polypeptides may be downregulated by a single method or by multiple methods; in either case, one or more compositions may be employed.

[0015] Methods for modulating drought tolerance in plants are also a feature of the invention, as are plants produced by such methods. For example, a method of modulating drought tolerance comprises: (a) selecting at least one ACC synthase gene (e.g., ACS6) to impact, thereby providing at least one desired ACC synthase gene; (b) introducing a mutant form (e.g., an antisense or sense configuration of at least one ACC synthase gene or subsequence thereof, an RNA silencing configuration of at least one ACC synthase gene or subsequence thereof, and the like) of the at least one desired ACC synthase gene into the plant and (c) expressing the mutant form, thereby modulating drought tolerance in the plant. In certain embodiments, the mutant gene is introduced by *Agrobacterium*-mediated transfer, electroporation, micro-projectile bombardment, a sexual cross or the like.

[0016] Detection of expression products is performed either qualitatively (by detecting presence or absence of one or more product of interest) or quantitatively (by monitoring the level of expression of one or more product of interest). In one embodiment, the expression product is an RNA expression product. Aspects of the invention optionally include monitoring an expression level of a nucleic acid, polypeptide or chemical (e.g., ACC, ethylene, etc.) as noted herein for detection of ACC synthase, ethylene production, drought tolerance, etc., in a plant or in a population of plants.

[0017] Kits which incorporate one or more of the nucleic acids noted above are also a feature of the invention. Such kits can include any of the above noted components and further include, e.g., instructions for use of the components in any of the methods noted herein, packaging materials and/or containers for holding the components. For example, a kit for detection of ACS expression levels in a plant includes at least one polynucleotide sequence comprising a nucleic acid sequence, where the nucleic acid sequence is, e.g., at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 99.5% or more, identical to SEQ ID NO: 3 or a subsequence thereof or a complement thereof. The subsequence may be SEQ ID No. 1 or 2. In a further embodiment, the kit includes instructional materials for the use of the at least one polynucleotide sequence to modulate drought tolerance in a plant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. **1** provides details of a plasmid (SEQ ID NO: 3) comprising a hairpin construct. Full sequence of the described plasmid is provided in SEQ ID NO: 3. A ubiquitin promoter (UBI1ZM PRO) drives expression of the hairpin, which comprises TR3 and TR4 (SEQ ID NOS: 1 and 2).

[0019] FIG. **2** shows the yield of transformed plants under flowering stress in Season 1, Environment 1. Each bar represents a separate transformation event. Average yield of transgene-negative segregants is shown (139 bu/a) as control (CN). A total of 74% of the events yielded nominally more than the control plants. Plants representing 18 transgenic events outyielded the control at $P<0.10$.

[0020] FIG. **3** shows the yield of transformed plants of the invention under grain-fill stress in Season 1, Environment 2. Each bar represents a separate transformation event. Average yield of transgene-negative segregants is shown (176 bu/a) as control (CN). Thirteen events out-yielded the CN at $P<0.10$. Of these, eight had also shown significant improvement under flowering stress.

[0021] FIG. **4** shows the yield, as a percent of control, of transformed plants of the invention (indicated by a circle), as well as plants transformed using an alternative ACS6 down-regulation vector (indicated by a square) under grain fill stress in Season 1, Environment 3. Each data point represents a separate transformation event. NS=not statistically significant. SIG=statistically significant. The control plants are bulked transgene-negative segregants. As can be seen, 64% of the events of the invention had significantly superior yield; only 17% of the alternative ACS6 down-regulation events had significantly superior yield, relative to the control.

[0022] FIG. **5** shows the yield, as a percent of control, of transformed plants of the invention (indicated by a circle), as well as plants transformed using an alternative ACS6 down-regulation vector (indicated by a square) under rain-fed conditions in Season 1, Environment 4. Each data point represents a separate transformation event. NS=not significant. The control plants are bulked transgene-negative segregants. As can be seen, all points exhibiting statistically significant increases in yield represent events of the invention disclosed herein. In addition, all points exhibiting statistically significant decreases in yield are events containing the alternative ACS6 down-regulation vector.

[0023] FIG. **6** is a schematic of a representative expression cassette of the invention.

[0024] FIG. **7** is an alignment of rice ACS6 coding sequence with the TR4 hairpin truncation (SEQ ID NO: 2).

[0025] FIG. **8** is an alignment of maize ACS6 and rice ACS6 sequences.

[0026] FIG. **9** shows grain yield (bushels/acre) of events in Background 1 in Season 2.

[0027] FIG. **10** shows grain yield (bushels/acre) of events in Background 1 in Season 3.

[0028] FIG. **11** shows plant height (inches) of events in Background 1 in Season 3.

[0029] FIG. **12** shows grain yield (bushels/acre) and plant height (in inches) of events in Backgrounds 2 and 3 in Season 3.

[0030] FIG. **13** shows grain yield (bushels/acre) of events in Backgrounds 2 and 3 across three water treatments and four testers in Season 4.

[0031] FIG. **14** provides an alignment of amino acid sequences of ZmACS6 and ZmACS3.

[0032] FIG. **15** provides an alignment of coding sequences of ZmACS6 and ZmACS3.

[0033] FIG. **16** provides an alignment of TR3 (SEQ ID NO: 1) with ACS3.

[0034] FIG. **17** shows ACC levels for four events in transgenic and control root tissues at maize growth stage VT.

[0035] FIG. **18** provides quantitative rtPCR data indicating reduced expression of ACS6 in root tissue of maize seedlings transgenic for one of ten ACS downregulation events.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0036] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

[0037] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not limiting. The following is presented by way of illustration and is not intended to limit the scope of the invention.

[0038] The present invention now will be described more fully hereinafter with reference to the accompanying drawings and other illustrative non-limiting embodiments.

[0039] Many modifications and other embodiments of the invention set forth herein are within the scope of the claimed invention based on the benefit of the teachings in the present descriptions and the associated drawings. Therefore, it is to be understood that the invention is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

[0040] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of agronomy, botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art.

[0041] Units, prefixes and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range. Amino acids may be referred to herein either by their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. The terms defined below are more fully defined by reference to the specification as a whole.

[0042] In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

[0043] By "microbe" is meant any microorganism (including both eukaryotic and prokaryotic microorganisms), such as fungi, yeast, bacteria, actinomycetes, algae and protozoa, as well as other unicellular structures.

[0044] By "amplified" is meant the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification systems include the polymerase chain reaction (PCR) system, ligase chain reaction (LCR) system, nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario), Q-Beta Replicase systems, transcription-based amplification system (TAS) and strand displacement amplification (SDA). See, e.g., *Diagnostic Molecular Microbiology: Principles and Applications*, Persing, et al., eds., American Society for Microbiology, Washington, D.C. (1993). The product of amplification is termed an amplicon.

[0045] The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids that encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine; one exception is *Micrococcus rubens*, for which GTG is the methionine codon (Ishizuka, et al., (1993) *J. Gen. Microbiol.* 139:425-32)) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid is implicit in each described polypeptide sequence and incorporated herein by reference.

[0046] As to amino acid sequences, one of skill will recognize that individual substitution, deletion or addition to a nucleic acid, peptide, polypeptide or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" when the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, for example, any number of amino acid residues selected from the group of integers consisting of from 1 to 15, such as 1, 2, 3, 4, 5, 7 or 10, can be so altered. Conservatively modified variants typically provide biological activity similar to that of the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80% or 90%, preferably 60-90% of the binding of the native protein for its native substrate. Conser-

vative substitution tables providing functionally similar amino acids are well known in the art.

[0047] The following six groups each contain amino acids that are conservative substitutions for one another:

[0048] 1) Alanine (A), Serine (S), Threonine (T);

[0049] 2) Aspartic acid (D), Glutamic acid (E);

[0050] 3) Asparagine (N), Glutamine (Q);

[0051] 4) Arginine (R), Lysine (K);

[0052] 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V) and

[0053] 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton, Proteins, W. H. Freeman and Co. (1984).

[0054] As used herein, "consisting essentially of" means the inclusion of additional sequences to an object polynucleotide where the additional sequences do not selectively hybridize, under stringent hybridization conditions, to the same cDNA as does the original object polynucleotide and where the hybridization conditions include a wash step in 0.1×SSC and 0.1% sodium dodecyl sulfate at 65° C. Generally, additional sequence or sequences do not materially affect the basic and novel characteristics of the claimed invention, e.g. down-regulation of ACS6. For example, in an embodiment, additional sequences may be included at the 5' or 3' end of the hairpin structure without materially affecting the RNA interference function of the construct.

[0055] The term "construct" is used to refer generally to an artificial combination of polynucleotide sequences, i.e. a combination which does not occur in nature, normally comprising one or more regulatory elements and one or more coding sequences. The term may include reference to expression cassettes and/or vector sequences, as is appropriate for the context.

[0056] A "control" or "control plant" or "control plant cell" provides a reference point for measuring changes in phenotype of a subject plant or plant cell in which genetic alteration, such as transformation, has been effected as to a gene of interest. A subject plant or plant cell may be descended from a plant or cell so altered and will comprise the alteration.

[0057] A control plant or plant cell may comprise, for example: (a) a wild-type plant or cell, i.e., of the same genotype as the starting material for the genetic alteration which resulted in the subject plant or cell; (b) a plant or plant cell of the same genotype as the starting material but which has been transformed with a null construct (i.e., with a construct which has no known effect on the trait of interest, such as a construct comprising a marker gene); (c) a plant or plant cell which is a non-transformed segregant among progeny of a subject plant or plant cell; (d) a plant or plant cell genetically identical to the subject plant or plant cell but which is not exposed to conditions or stimuli that would induce expression of the gene of interest or (e) the subject plant or plant cell itself, under conditions in which the gene of interest is not expressed. A control plant may also be a plant transformed with an alternative ACS6 down-regulation construct.

[0058] By "encoding" or "encoded," with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the "universal" genetic code. However, variants of the universal code, such as is present in some plant, animal and fungal mitochondria, the bacterium *Mycoplasma capricolumn* (Yamao, et al., (1985) *Proc. Natl. Acad. Sci. USA* 82:2306-9) or the ciliate Macronucleus, may be used when the nucleic acid is expressed using these organisms.

[0059] When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host in which the nucleic acid is to be expressed. For example, although nucleic acid sequences may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledonous plants or dicotyledonous plants (see Murray, et al., (1989) *Nucleic Acids Res.* 17:477-98, and herein incorporated by reference). Thus, the maize preferred codon for a particular amino acid might be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray, et al., supra.

[0060] By "flowering stress" is meant that water is withheld from plants such that drought stress occurs at or around the time of anthesis.

[0061] By "grain fill stress" is meant that water is withheld from plants such that drought stress occurs during the time when seeds are accumulating storage products (carbohydrates, protein and/or oil).

[0062] By "rain-fed conditions" is meant that water is neither deliberately withheld nor artificially supplemented.

[0063] By "well-watered conditions" is meant that water available to the plant is generally adequate for optimum growth.

[0064] Drought stress conditions for maize may be controlled to result in a targeted yield reduction. For example, a 20%, 30%, 40%, 50%, 60%, 70% or greater reduction in yield of control plants can be accomplished by providing measured amounts of water during specific phases of plant development.

[0065] As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from its original form by deliberate human intervention.

[0066] By "host cell" is meant a cell which comprises a heterologous nucleic acid sequence of the invention. Host cells may be prokaryotic cells such as *E. coli* or eukaryotic cells such as yeast, insect, plant, amphibian or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells, including but not limited to maize, sorghum, sunflower, soybean, wheat, alfalfa, rice, cotton, canola, barley, millet, sugarcane, turfgrass and tomato. A particularly preferred monocotyledonous host cell is a maize host cell.

[0067] The term "hybridization complex" includes reference to a duplex nucleic acid structure formed by two single-stranded nucleic acid sequences selectively hybridized with each other.

[0068] The term "down-regulate" and its forms, e.g. down-regulation, refers to a reduction which may be partial or complete. For example, down-regulation of an ACS polynucleotide in a plant or cell encompasses a reduction in expression to a level that is 99%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% or 0% of the expression level of the corresponding ACS polynucleotide in a control plant or cell.

[0069] The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon or transiently expressed (e.g., transfected mRNA).

[0070] The term "isolated" refers to material, such as a nucleic acid or a protein, which is substantially or essentially free from components which normally accompany or interact with it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment. Nucleic acids which are "isolated", as defined herein, are also referred to as "heterologous" nucleic is acids.

[0071] As used herein the term "modulation of ACC synthase activity" shall be interpreted to mean any change in an ACC synthase biological activity, which can include an altered level of ACC synthase present in a plant cell, altered efficacy of the enzyme or any other means which affects one or more of the biological properties of ACC synthase in relation to its role in converting S-adenosylmethionine to ACC in the formation of ethylene. Accordingly, "inhibition of ACC synthase activity" encompasses a reduction in the efficacy of the enzyme or a reduction in the level of ACC synthase present in a plant cell, for example, due to a reduction in the expression of an ACC synthase gene.

[0072] In other embodiments, expression of a downregulation construct described herein could modulate other steps along the ethylene synthesis pathway to improve plant yield or abiotic stress tolerance of a plant. For example, the rate of conversion of SAM to polyamines could be increased or the level or activity of ACC oxidase could be decreased or the level or activity of SAM could be increased or some combination of these and/or other modifications in the ethylene synthesis pathway could occur as a result of the genetic modulation described herein. While not wishing to be bound by any theory, it is postulated that modification of one or more steps towards ethylene synthesis results in decreased ethylene activity. In any event, the invention is directed to increasing plant yield in optimum conditions, as well as improving performance under abiotic stress conditions, by modulating expression of an ACC synthase gene, regardless of the precise effect of that modulation on the ethylene synthesis pathway, ethylene production or ethylene activity.

[0073] The term "nitrogen utilization efficiency" (NUE) refers to physiological processes of uptake and/or assimilation of nitrogen and/or the subsequent remobilization and reutilization of accumulated nitrogen reserves. Improved NUE refers to enhancement of these processes relative to a control plant. Plants in which NUE is improved may be more productive than control plants under comparable conditions of ample nitrogen availability and/or may maintain productivity under significantly reduced nitrogen availability. Improving NUE, particularly in maize, would increase harvestable yield per unit of input nitrogen fertilizer, both in developing nations where access to nitrogen fertilizer is limited and in developed nations where the level of nitrogen use is high. Improved NUE reduces on-farm input costs, decreases dependence on the non-renewable energy sources required for nitrogen fertilizer production and diminishes the environmental impact of nitrogen fertilizer manufacturing and agricultural use. Improved NUE may be reflected in one or more attributes such as increased biomass, increased grain yield, increased harvest index, increased photosynthetic rates and increased tolerance to biotic or abiotic stress. These attributes may reflect or result in changes including a modulation of root development, shoot and leaf development and/or reproductive tissue development. By "modulating root development" is intended any alteration in the development of the plant root when compared to a control plant. Such alterations in root development include, but are not limited to, alterations in the growth rate of the primary root, the fresh root weight, the extent of lateral and adventitious root formation, the vasculature system, meristem development or radial expansion. Furthermore, higher root biomass production may affect production of compounds synthesized by root cells or transgenic root cells or cell cultures of said transgenic root cells. Methods of measuring developmental alterations in the root system are known in the art. See, for example, US Patent Application Publication Number 2003/0074698 and Werner, et al., (2001) *PNAS* 18:10487-10492, both of which are herein incorporated by reference.

[0074] Reducing activity of at least one ACC synthase in a plant can improve the nitrogen stress tolerance of the plant. Such plants may exhibit maintenance of productivity with significantly less nitrogen fertilizer input and/or exhibit enhanced uptake and assimilation of nitrogen fertilizer and/or exhibit altered remobilization and reutilization of accumulated nitrogen reserves or exhibit any combination of such characteristics. In addition to an overall increase in yield, the improvement of nitrogen stress tolerance through the inhibition of ACC synthase can also result in increased root mass and/or length, increased ear, leaf, seed and/or endosperm size and/or improved standability. Accordingly, in some embodiments, the methods further comprise growing said plants under nitrogen limiting conditions and optionally selecting those plants exhibiting greater tolerance to the low nitrogen levels.

[0075] Further, methods and compositions are provided for improving yield under abiotic stress, which include evaluating the environmental conditions of an area of cultivation for abiotic stressors (e.g., low nitrogen levels in the soil) and growing plants having reduced ethylene synthesis, which in some embodiments is due to reduced activity of at least one ACC synthase, in stressful environments.

[0076] The term "low nitrogen conditions" or "nitrogen limiting conditions" as used herein shall be interpreted to mean any environmental condition in which plant-available nitrogen is less than would be optimal for expression of maximum yield potential.

[0077] As used herein, "nucleic acid" includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids).

[0078] By "nucleic acid library" is meant a collection of isolated DNA or RNA molecules which comprise and substantially represent the entire transcribed fraction of a genome of a specified organism. Construction of exemplary nucleic acid libraries, such as genomic and cDNA libraries, is taught in standard molecular biology references such as Berger and Kimmel, (1987) *Guide To Molecular Cloning Techniques*, from the series *Methods in Enzymology*, vol. 152, Academic Press, Inc., San Diego, Calif.; Sambrook, et al., (1989) *Molecular Cloning: A Laboratory Manual, 2nd* ed., vols. 1-3 and *Current Protocols in Molecular Biology*, Ausubel, et al., eds, Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1994 Supplement).

[0079] As used herein "operably linked" includes reference to a functional linkage between a first sequence, such as a promoter, and a second sequence, wherein the promoter sequence initiates and mediates transcription of the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

[0080] As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds and plant cells and progeny of same. Plant cell, as used herein includes, without limitation, cells in or from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen and microspores. The class of plants which can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants including species from the genera: *Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Avena, Hordeum, Secale, Allium* and *Triticum*. A particularly preferred plant is *Zea mays*.

[0081] As used herein, "yield" may include reference to bushels per acre of a grain crop at harvest, as adjusted for grain moisture (typically 15% for maize, for example) and/or the volume of biomass generated (e.g. for forage crops such as alfalfa, maize for silage and any species grown for biofuel production). Biomass is measured as the weight of harvestable plant material generated.

[0082] As used herein, "polynucleotide" includes reference to a deoxyribopolynucleotide, ribopolynucleotide or analogs thereof that have the essential nature of a natural ribonucleotide in that they hybridize, under stringent hybridization conditions, to substantially the same nucleotide sequence as do the naturally occurring polynucleotides and/or allow translation into the same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can be full-length or a subsequence of a native or heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including inter alia, simple and complex cells.

[0083] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0084] As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and/or other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses and bacteria which comprise genes expressed in plant cells such as *Agrobacterium* or *Rhizobium*.

[0085] The term "ACS polypeptide" refers to one or more amino acid sequences of an ACS enzyme. The term is also inclusive of fragments, variants, homologs, alleles or precursors (e.g., preproproteins or proproteins) thereof. An "ACS protein" comprises an ACS polypeptide.

[0086] As used herein "recombinant" includes reference to a cell or vector that has been modified by the introduction of a heterologous nucleic acid or a cell that is derived from a cell so modified and maintains the modification. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all, as a result of deliberate human intervention or may have reduced or eliminated expression of a native gene. In certain examples, recombinant cells exhibit reduced expression of one or more targeted genes or a reduced level or activity of a polypeptide of interest, relative to the non-recombinant cell. The term "recombinant" as used herein does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

[0087] As used herein, a "recombinant expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements, which permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed and a promoter.

[0088] The terms "residue" and "amino acid residue" and "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited,

may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

[0089] The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 40% sequence identity, often 60-90% sequence identity and may have 100% sequence identity (i.e., are complementary) with each other.

[0090] The terms "stringent conditions" or "stringent hybridization conditions" include reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which can be up to 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Optimally, the probe is approximately 500 nucleotides in length, but can vary greatly in length from less than 500 nucleotides to equal to the entire length of the target sequence.

[0091] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide or Denhardt's. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37° C. and a wash in 1× to 2×SSC (20×SSC=3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55° C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37° C. and a wash in 0.5× to 1×SSC at 55 to 60° C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37° C. and a wash in 0.1×SSC at 60 to 65° C. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, (1984) *Anal. Biochem.,* 138:267-84: T_m=81.5° C.+16.6 (log M)+0.41 (% GC)−0.61 (% form)−500/L; where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1° C. for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the T_m can be decreased 10° C. Generally, stringent conditions are selected to be about 5° C. lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3 or 4° C. lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9 or 10° C. lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or 20° C. lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45° C. (aqueous solution) or 32° C. (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, New York (1993) and *Current Protocols in Molecular Biology*, chapter 2, Ausubel, et al., eds, Greene Publishing and Wiley-Interscience, New York (1995).

[0092] As used herein, "transgenic plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition or spontaneous mutation.

[0093] As used herein, "vector" includes reference to a nucleic acid used in transfection of a host cell and into which can be inserted a polynucleotide. Vectors are often replicons. Expression vectors permit transcription of a nucleic acid inserted therein.

[0094] The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides or polypeptides: (a) "reference sequence," (b) "comparison window," (c) "sequence identity," (d) "percentage of sequence identity" and (e) "substantial identity."

[0095] As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence or the complete cDNA or gene sequence.

[0096] As used herein, "comparison window" includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or

deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100 or more nucleotides. Those of skill in the art understand that to avoid inference of inappropriately high similarity to a reference sequence, a gap penalty is typically introduced and is subtracted from the number of matches.

[0097] Methods of alignment of nucleotide and amino acid sequences for comparison are well known in the art, such as the local homology algorithm (BESTFIT) of Smith and Waterman, (1981) *Adv. Appl. Math* 2:482, which may conduct optimal alignment of sequences for comparison; the homology alignment algorithm (GAP) of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443-53; the search for similarity method (Tfasta and Fasta) of Pearson and Lipman, (1988) *Proc. Natl. Acad. Sci. USA* 85:2444 and computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, Calif., GAP, BESTFIT, BLAST, FASTA and TFASTA in the Wisconsin Genetics Software Package®, Version 8 (available from Genetics Computer Group (GCG® programs, Accelrys, Inc., San Diego, Calif.)). The CLUSTAL program is well described by Higgins and Sharp, (1988) *Gene* 73:237-44; Higgins and Sharp, (1989) *CABIOS* 5:151-3; Corpet, et al., (1988) *Nucleic Acids Res.* 16:10881-90; Huang, et al., (1992) *Computer Applications in the Biosciences* 8:155-65 and Pearson, et al., (1994) *Meth. Mol. Biol.* 24:307-31. The preferred program to use for optimal global alignment of multiple sequences is PileUp (Feng and Doolittle, (1987) *J. Mol. Evol.,* 25:351-60 which is similar to the method described by Higgins and Sharp, (1989) *CABIOS* 5:151-53 and hereby incorporated by reference). The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology*, Chapter 19, Ausubel et al., eds., Greene Publishing and Wiley-Interscience, New York (1995).

[0098] Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package® are 8 and 2, respectively. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 100. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 or greater. GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package® is BLOSUM62 (see, Henikoff and Henikoff, (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

[0099] Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the BLAST 2.0 suite of programs using default parameters (Altschul, et al., (1997) *Nucleic Acids Res.* 25:3389-402).

[0100] As those of ordinary skill in the art will understand, BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences, which may be homopolymeric tracts, short-period repeats or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, (1993) *Comput. Chem.* 17:149-63) and XNU (Claverie and States, (1993) *Comput. Chem.* 17:191-201) low-complexity filters can be employed alone or in combination.

[0101] As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences, which differ by such conservative substitutions, are said to have "sequence similarity" or "similarity." Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, (1988) *Computer Applic. Biol. Sci.* 4:11-17, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif., USA).

[0102] As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0103] The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has between 50-100% sequence identity, such as at least

50% 60%, 70%, 80%, 90% or 95% sequence identity, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 55-100%, such as 55%, 60%, 70%, 80%, 90% or 95%.

[0104] Another indication that nucleotide sequences are substantially identical is that two molecules hybridize to each other under stringent conditions. The degeneracy of the genetic code allows for many nucleic acid substitutions that lead to variety in the nucleotide sequence that code for the same amino acid, hence it is possible that two DNA sequences could code for the same polypeptide but not hybridize to each other under stringent conditions. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

[0105] The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with between 55-100% sequence identity to a reference sequence, such as 55%, 60%, 70%, 80%, 85%, 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, supra. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. In addition, a peptide can be substantially identical to a second peptide when they differ by a non-conservative change if the epitope that the antibody recognizes is substantially identical. Peptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes.

Construction of Nucleic Acids

[0106] The isolated nucleic acids can be made using: (a) standard recombinant methods, (b) synthetic techniques or (c) combinations thereof. In some embodiments, the polynucleotides will be cloned, amplified or otherwise constructed from plants, fungi or bacteria.

[0107] A nucleic acid, excluding the polynucleotide sequence, is optionally a vector, adapter or linker for cloning and/or expression of a polynucleotide. Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide or to improve the introduction of the polynucleotide into a cell. For example one may use recombination sites, such as FRT sites, for creation and isolation of the polynucleotides of the invention, as disclosed in US Patent Application Publication Number 2008/0202505. Examples of recombination sites are known in the art and include FRT sites (See, for example, Schlake and Bode, (1994) *Biochemistry* 33:12746-12751; Huang, et al., (1991) *Nucleic Acids Research* 19:443-448; Sadowski, (1995) In Progress in Nucleic Acid Research and Molecular Biology vol. 51, pp. 53-91; Cox, (1989) In Mobile DNA, Berg and Howe, (eds) American Society of Microbiology, Washington D.C., pp. 116-670; Umlauf and Cox, (1988) *The EMBO Journal* 7:1845-1852; Buchholz, et al., (1996) *Nucleic Acids Research* 24:3118-3119; Kilby, et al., (1993) *Trends Genet.* 9:413-421; Rossant and Geagy, (1995) *Nat. Med.* 1:592-594; Albert, et al., (1995) *The Plant Journal* 7:649-659; Bayley, et al., (1992) *Plant Mol. Biol.* 18:353-361; Odell, et al., (1990) *Mol. Gen. Genet.* 223:369-378 and Dale and Ow, (1991) *Proc. Natl. Acad. Sci. USA* 88:10558-105620, all of which are herein incorporated by reference); Lox (Albert, et al., (1995) *Plant J.* 7:649-659; Qui, et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:1706-1710; Stuurman, et al., (1996) *Plant Mol. Biol.* 32:901-913; Odell, et al., (1990) *Mol. Gen. Genet.* 223:369-378; Dale, et al., (1990) *Gene* 91:79-85 and Bayley, et al., (1992) *Plant Mol. Biol.* 18:353-361; Vega, et al., (2008) *Plant Mol. Biol.* 66(6):587-598).

[0108] Site-specific recombinases like FLP cleave and religate DNA at specific target sequences, resulting in a precisely defined recombination between two identical sites. To function, the system needs the recombination sites and the recombinase. No auxiliary factors are needed. Thus, the entire system can be inserted into and function in plant cells. Engineering FLP/FRT sites within, or adjacent to, the hairpin structure may facilitate excision of selectable markers and other vector backbone sequence from a host cell.

[0109] Use of cloning vectors, expression vectors, adapters and linkers is well known in the art. Exemplary nucleic acids include such vectors as: M13, lambda ZAP Express, lambda ZAP II, lambda gt10, lambda gt11, pBK-CMV, pBK-RSV, pBluescript II, lambda DASH II, lambda EMBL 3, lambda EMBL 4, pWE15, SuperCos 1, SurfZap, Uni-ZAP, pBC, pBS+/−, pSG5, pBK, pCR-Script, pET, pSPUTK, p3'SS, pGEM, pSK+/−, pGEX, pSPORTI and II, pOPRSVI CAT, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pOG44, pOG45, pFRTβGAL, pNEOβGAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, pRS416, lambda MOSSlox and lambda MOSElox. Optional vectors for the present invention, include but are not limited to, lambda ZAP II and pGEX. For a description of various nucleic acids see, e.g., Stratagene Cloning Systems, Catalogs 1995, 1996, 1997 (La Jolla, Calif.) and Amersham Life Sciences, Inc, Catalog '97 (Arlington Heights, Ill.).

Synthetic Methods for Constructing Nucleic Acids

[0110] The isolated nucleic acids can also be prepared by direct chemical synthesis as known in the art. Chemical synthesis generally produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. Longer sequences may be obtained by the ligation of shorter sequences.

UTRs and Codon Preference

[0111] In general, translational efficiency has been found to be regulated by specific sequence elements in the 5' non-coding or untranslated region (5' UTR) of the RNA. Positive sequence motifs include translational initiation consensus sequences (Kozak, (1987) *Nucleic Acids Res.* 15:8125) and the 5<G>7 methyl GpppG RNA cap structure (Drummond, et al., (1985) *Nucleic Acids Res.* 13:7375). Negative elements

include stable intramolecular 5' UTR stem-loop structures (Muesing, et al., (1987) *Cell* 48:691) and AUG sequences or short open reading frames preceded by an appropriate AUG in the 5' UTR (Kozak, supra, Rao, et al., (1988) *Mol. and Cell. Biol.* 8:284). Accordingly, the present invention provides 5' and/or 3' UTR regions for modulation of translation of heterologous coding sequences.

[0112] Further, the polypeptide-encoding segments of the polynucleotides can be modified to alter codon usage. Altered codon usage can be employed to alter translational efficiency and/or to optimize the coding sequence for expression in a desired host or to optimize the codon usage in a heterologous sequence for expression in maize. Codon usage in the coding regions of the polynucleotides can be analyzed statistically using commercially available software packages such as "Codon Preference" available from the University of Wisconsin Genetics Computer Group. See, Devereaux, et al., (1984) *Nucleic Acids Res.* 12:387-395) or MacVector 4.1 (Eastman Kodak Co., New Haven, Conn.). The number of polynucleotides (3 nucleotides per amino acid) that can be used to determine a codon usage frequency can be any integer from 3 to the number of polynucleotides tested. Optionally, the polynucleotides will be full-length sequences. An exemplary number of sequences for statistical analysis can be at least 1, 5, 10, 20, 50 or 100.

Recombinant Expression Cassettes

[0113] The present invention further provides recombinant expression cassettes comprising a nucleic acid. A recombinant expression cassette will typically comprise a polynucleotide operably linked to transcriptional initiation regulatory sequences which will direct the transcription of the polynucleotide in the intended host cell, such as tissues of a transformed plant.

[0114] For example, plant expression vectors may include: (1) a cloned plant gene under the transcriptional control of 5' and 3' regulatory sequences and (2) a dominant selectable marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (e.g., one conferring inducible or constitutive, environmentally- or developmentally-regulated or cell- or tissue-specific/preferred expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site and/or a polyadenylation signal.

[0115] A plant promoter fragment can be employed which will direct expression of a polynucleotide in all, or nearly all, tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the 1'- or 2'-promoter derived from T-DNA of *Agrobacterium tumefaciens*, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Pat. No. 5,683,439), the Nos promoter, the rubisco promoter, the GRP1-8 promoter, the 35S promoter from cauliflower mosaic virus (CaMV), as described in Odell, et al., (1985) *Nature* 313:810-2; rice actin (McElroy, et al., (1990) *Plant Cell* 163-171); ubiquitin (Christensen, et al., (1992) *Plant Mol. Biol.* 12:619-632 and Christensen, et al., (1992) *Plant Mol. Biol.* 18:675-89); pEMU (Last, et al., (1991) *Theor. Appl. Genet.* 81:581-8); MAS (Velten, et al., (1984) *EMBO J.* 3:2723-30) and maize H3 histone (Lepetit, et al., (1992) *Mol. Gen. Genet.* 231:276-85 and Atanassvoa, et al., (1992) *Plant Journal* 2(3):291-300); ALS promoter, as described in PCT Application Number WO 1996/30530 and other transcription initiation regions from various plant genes known to those of skill in the art.

[0116] Tissue preferred, cell type preferred, developmentally regulated and inducible promoters are examples of "non-constitutive" promoters.

[0117] Tissue-preferred promoters can be utilized to target expression within a particular plant tissue. By "tissue-preferred" is intended to mean that expression is predominantly in a particular tissue, albeit not necessarily exclusively in that tissue. Examples include promoters that preferentially initiate transcription in leaves, roots, seeds, endosperm, fibers, xylem vessels, tracheids or sclerenchyma. Certain tissue-preferred promoters may drive expression only in photosynthetic ("green") tissue. Tissue-preferred promoters include Yamamoto, et al., (1997) *Plant J.* 12(2):255-265; Kawamata, et al., (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen, et al., (1997) *Mol. Gen. Genet.* 255(3):337-353; Russell, et al., (1997) *Transgenic Res.* 6(2):157-168; Rinehart, et al., (1996) *Plant Physiol.* 112(3):1331-1351; Van Camp, et al., (1996) *Plant Physiol.* 112(2):525-535; Canevascini, et al., (1996) *Plant Physiol.* 112(2):513-525; Yamamoto, et al., (1995) *Plant Cell Physiol.* 35(5):773-778; Lam, (1995) *Results Probl. Cell Differ.* 20:181-196; Orozco, et al., (1993) *Plant Mol. Biol.* 23(6):1129-1138; Matsuoka, et al., (1993) *Proc Natl. Acad. Sci. USA* 90(20):9586-9590; the maize glb1 promoter (GenBank L22344) and Guevara-Garcia, et al., (1993) *Plant J.* 5(3):595-505. Such promoters can be modified, if necessary, for weak expression. See, also, US Patent Application Number 2003/0074698, herein incorporated by reference.

[0118] Shoot-preferred promoters include, shoot meristem-preferred promoters such as promoters disclosed in Weigal, et al., (1992) *Cell* 69:853-859; Accession Number AJ131822; Accession Number Z71981; Accession Number AF059870, the ZAP promoter (U.S. patent application Ser. No. 10/387,937), the maize tb1 promoter (Wang, et al., (1999) *Nature* 398:236-239 and shoot-preferred promoters disclosed in McAvoy, et al., (2003) *Acta Hort. (ISHS)* 625:379-385.

[0119] Root-preferred promoters are known and can be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire, et al., (1992) *Plant Mol. Biol.* 20(2):207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner, (1991) *Plant Cell* 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger, et al., (1990) *Plant Mol. Biol.* 15(3):533-553 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*) and Miao, et al., (1991) *Plant Cell* 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also, Bogusz, et al., (1990) *Plant Cell* 2(7):633-651; Leach and Aoyagi, (1991) *Plant Science* (Limerick) 79(1):69-76); Teeri, et al., (1989) *EMBO J.* 8(2):353-350. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster, et al., (1995) *Plant Mol. Biol.* 29(5):759-772); rolB promoter (Capana, et al., (1995) *Plant Mol. Biol.* 25(5):681-691 and the CRWAQ81 root-preferred promoter with the ADH first intron (U.S. Pat. No. 7,411,112). See also, U.S. Pat. Nos. 5,837,876; 5,750,386; 5,633,363; 5,559,252; 5,501,836; 5,110,732 and 5,023,179.

[0120] A "cell type"-specific or cell type-preferred promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves

or mesophyll cells. A mesophyllic cell preferred promoter includes, but is not limited to, known phosphoenopyruvate decarboxylase (PEPC) promoters or putative PEPC promoters from any number of species, for example, *Zea mays, Oryza sativa, Arabidopsis thaliana, Glycine max* or *Sorghum* bicolor. Examples include *Zea mays* PEPC of GenBank Accession Number gi:116268332_HTG AC190686 and gCAT GSS composite sequence; *Oryza sativa* PEPC of GenBank Accession Number gi|20804452|dbj|AP003052.3|; *Arabidopsis thaliana* PEPC of GenBank Accession Number gi|5541653|dbj|AP000370.1|AP000370; gi:7769847 or gi|20198070|gb|AC007087.7; *Glycine max* (GSS contigs) or *Sorghum* bicolor (JGI assembly scaffold_832, 89230 bp., JGI assembly scaffold_1632, (1997) *Plant J.* 12(2):255-265; Kwon, et al., (1995) *Plant Physiol.* 105:357-67; Yamamoto, et al., (1995) *Plant Cell Physiol.* 35(5):773-778; Gotor, et al., (1993) *Plant J.* 3:509-18; Orozco, et al., (1993) *Plant Mol. Biol.* 23(6):1129-1138; Baszczynski, et al., (1988) *Nucl. Acid Res.* 16:5732; Mitra, et al., (1995) *Plant Molecular Biology* 26:35-93; Kayaya, et al., (1995) *Molecular and General Genetics* 258:668-675 and Matsuoka, et al., (1993) *Proc. Natl. Acad. Sci. USA* 90(20):9586-9590.

[0121] The plant promoter may be under more precise environmental control, e.g. the promoter may initiate transcription of an operably-linked gene in response to an external stimulus. Such promoters are referred to here as "inducible" promoters. Environmental conditions that may effect transcription by inducible promoters include pathogen attack, anaerobic conditions or the presence of light. Examples of inducible promoters are the Adh1 promoter, which is inducible by hypoxia or cold stress; the Hsp70 promoter, which is inducible by heat stress; the PPDK promoter, which is inducible by light and abiotic-stress-inducible promoters rab17 (Vilardell, et al., (1991) *Plant Mol. Biol.* 17(5):985-993); rd29a (Yamaguchi-Shinozaki, et al., (1993) *Mol. Gen. Genet.* 236:331-340) and KT250 (US Patent Publication Number 2009/0229014); see also, US Patent Publication Number 2004/0123347.

[0122] A developmentally regulated promoter may have both a temporal and a spatial limitation, for example, a promoter that drives expression in specific tissue types during pollen development or during inflorescence development. See, e.g., US Patent Publication Numbers 2007/0234444 and 2009/0094713. Another example is a senescence regulated promoter, such as SAM22 (Crowell, et al., (1992) *Plant Mol. Biol.* 18:559-566); see also, U.S. Pat. No. 5,589,052.

[0123] Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

[0124] If polypeptide expression is desired, a polyadenylation region is often included at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from a variety of plant genes or from T-DNA. The sequence to be added can be derived from, for example, the nopaline synthase or octopine synthase genes or alternatively from another plant gene or less preferably from any other eukaryotic gene. Examples of such regulatory elements include, but are not limited to, 3' termination and/or polyadenylation regions such as those of the *Agrobacterium tumefaciens* nopaline synthase (nos) gene (Bevan, et al., (1983) *Nucleic Acids Res.* 12:369-85); the potato proteinase inhibitor II (PI-NII) gene (Keil, et al., (1986) *Nucleic Acids Res.* 14:5641-50 and An, et al., (1989) *Plant Cell* 1:115-22) and the CaMV 19S gene (Mogen, et al., (1990) *Plant Cell* 2:1261-72).

[0125] An intron sequence can be added to the 5' untranslated region or the coding sequence or the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol; for example, the maize Adh1 and Bz1 introns (Callis, et al., (1987) *Genes Dev.* 1:1183-1200). Inclusion of a spliceable intron in the transcription unit in expression constructs has been shown to increase gene expression at both the mRNA and protein levels (if applicable) up to 1000-fold (Buchman and Berg, (1988) *Mol. Cell. Biol.* 8:4395-4405). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. For a review, see Simpson and Filipowicz, (1996) *Plant Mol. Biol.* 32:1-41.

[0126] Plant signal sequences include, but are not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell (Dratewka-Kos, et al., (1989) *J. Biol. Chem.* 264:4896-900), such as the *Nicotiana plumbaginifolia* extension gene (DeLoose, et al., (1991) *Gene* 99:95-100); signal peptides which target proteins to the vacuole, such as the sweet potato sporamin gene (Matsuka, et al., (1991) *Proc. Natl. Acad. Sci. USA* 88:834) and the barley lectin gene (Wilkins, et al., (1990) *Plant Cell,* 2:301-13); signal peptides which cause proteins to be secreted, such as that of PRIb (Lind, et al., (1992) *Plant Mol. Biol.* 18:47-53) or barley alpha amylase (BAA) (Rahmatullah, et al., (1989) *Plant Mol. Biol.* 12:119) or signal peptides which target proteins to the plastids such as that of rapeseed enoyl-Acp reductase (Verwaert, et al., (1994) *Plant Mol. Biol.* 26:189-202).

[0127] A vector comprising the sequences of a polynucleotide of the present invention will typically comprise a marker gene which confers a selectable phenotype on plant cells. The selectable marker gene may encode antibiotic resistance, with suitable genes including genes coding for resistance to the antibiotic spectinomycin (e.g., the aada gene), the streptomycin phosphotransferase (SPT) gene coding for streptomycin resistance, the neomycin phosphotransferase (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (HPT) gene coding for hygromycin resistance. Also useful are genes coding for resistance to herbicides which act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides which act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the bar gene) or other such genes known in the art. The bar gene encodes resistance to the herbicide basta and the ALS gene encodes resistance to the herbicide chlorsulfuron. Also useful are genes encoding resistance to glyphosate; see, for example, U.S. Pat. Nos. 7,462,481; 7,531,339; 7,405,075; 7,666,644; 7,622,641 and 7,714,188. Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers, et al., (1987), *Meth. Enzymol.* 153:253-77. These vectors are plant integrating vectors in that on transformation, the vectors integrate a portion of vector DNA into the genome of the host plant. Exemplary *A. tumefaciens* vectors useful herein are plasmids

pKYLX6 and pKYLX7 of Schardl, et al., (1987) *Gene* 61:1-11 and Berger, et al., (1989) *Proc. Natl. Acad. Sci. USA,* 86:8402-6. Another useful vector herein is plasmid pBI101.2, available from CLONTECH Laboratories, Inc. (Palo Alto, Calif.).

Expression of Sequences in Host Cells

[0128] One may express a polynucleotide in a recombinantly engineered cell such as bacteria, yeast, insect or preferably plant cell. The cell produces the polynucleotide in a non-natural condition (e.g., altered in quantity, composition, location and/or time), because it has been genetically altered through human intervention to do so.

[0129] It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a polynucleotide. No attempt will be made to describe in detail all the various methods known for expression in prokaryotes or eukaryotes.

[0130] In brief summary, the expression of isolated polynucleotides will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter, followed by incorporation into an expression vector. The vector can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences and promoters useful for regulation of the expression of the DNA. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a promoter such as ubiquitin to direct transcription, a ribosome binding site for translational initiation and a transcription/translation terminator. Constitutive promoters are classified as providing for a range of constitutive expression. Thus, some are weak constitutive promoters and others are strong constitutive promoters. See, for example, U.S. Pat. No. 6,504,083. Generally, by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By "low level" is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts to about /**1;500,000** transcripts. Conversely, a "strong promoter" drives expression of a coding sequence at a "high level" or about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts.

Expression in Prokaryotes

[0131] Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of *E. coli*; however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., (1977) *Nature* 198:1056), the tryptophan (trp) promoter system (Goeddel, et al., (1980) *Nucleic Acids Res.* 8:4057) and the lambda derived P L promoter and N-gene ribosome binding site (Shimatake, et al., (1981) *Nature* 292:128). The inclusion of selection markers in DNA vectors transfected in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline or chloramphenicol.

[0132] The vector is selected to allow introduction of the gene of interest into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein are available using *Bacillus* sp. and *Salmonella* (Palva, et al., (1983) *Gene* 22:229-35; Mosbach, et al., (1983) *Nature* 302: 543-5).

Expression in Eukaryotes

[0133] A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells are known to those of skill in the art. As explained briefly below, the present invention can be expressed in these eukaryotic systems. In some embodiments, transformed/transfected plant cells, as discussed infra, are employed as expression systems for production of the proteins of the instant invention.

[0134] Synthesis of heterologous proteins in yeast is well known. Sherman, et al., (1982) *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory is a well recognized work describing the various methods available to produce the protein in yeast. Two widely utilized yeasts for production of eukaryotic proteins are *Saccharomyces cerevisiae* and *Pichia pastoris*. Vectors, strains and protocols for expression in *Saccharomyces* and *Pichia* are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as promoters, including 3-phosphoglycerate kinase or alcohol oxidase and an origin of replication, termination sequences and the like as desired.

[0135] A protein, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates or the pellets. The monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

[0136] The sequences encoding proteins can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, insect or plant origin. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter (e.g., the CMV promoter, a HSV tk promoter or pgk (phosphoglycerate kinase) promoter), an enhancer (Queen, et al., (1986) *Immunol. Rev.* 89:49) and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site) and transcriptional terminator sequences. Other animal cells useful for production of proteins are available, for instance, from the American Type Culture Collection, P.O. Box 1549, Manassas, Va., USA, 20108.

[0137] As with yeast, when plant host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the potato pinII terminator (Keil et al., supra; An et al., supra). Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., *J. Virol.* 45:773-81 (1983)).

Plant Transformation Methods

[0138] Numerous methods for introducing foreign genes into plants are known and can be used to insert an ACS polynucleotide into a plant host, including biological and physical plant transformation protocols. See, e.g., Miki, et al., "Procedure for Introducing Foreign DNA into Plants," in

Methods in Plant Molecular Biology and Biotechnology, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pp. 67-88 (1993). The methods chosen vary with the host plant and include chemical transfection methods such as calcium phosphate, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch et al., *Science* 227:1229-31 (1985)), electroporation, micro-injection and biolistic bombardment.

[0139] Expression cassettes and vectors and in vitro culture methods for plant cell or tissue transformation and regeneration of plants are known and available. See, e.g., Gruber, et al., "Vectors for Plant Transformation," in *Methods in Plant Molecular Biology and Biotechnology*, supra, pp. 89-119.

[0140] The isolated polynucleotides or polypeptides may be introduced into the plant by one or more techniques typically used for direct delivery into cells. Such protocols may vary depending on the type of organism, cell, plant or plant cell, e.g., monocot or dicot, targeted for gene modification. Suitable methods of transforming plant cells include microinjection (Crossway, et al., (1986) *Biotechniques* 4:320-334 and U.S. Pat. No. 6,300,543), electroporation (Riggs, et al., (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606, direct gene transfer (Paszkowski et al., (1984) *EMBO J.* 3:2717-2722) and ballistic particle acceleration (see, for example, Sanford, et al., U.S. Pat. No. 4,945,050; WO 91/10725 and McCabe, et al., (1988) *Biotechnology* 6:923-926). Also see, Tomes, et al., "Direct DNA Transfer into Intact Plant Cells Via Microprojectile Bombardment". pp. 197-213 in *Plant Cell, Tissue and Organ Culture, Fundamental Methods*. eds. Gamborg and Phillips, Springer-Verlag Berlin Heidelberg New York, 1995; U.S. Pat. No. 5,736,369 (meristem); Weissinger, et al., (1988) *Ann. Rev. Genet.* 22:421-477; Sanford, et al., (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou, et al., (1988) *Plant Physiol.* 87:671-674 (soybean); Datta, et al., (1990) *Biotechnology* 8:736-740 (rice); Klein, et al., (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein, et al., (1988) *Biotechnology* 6:559-563 (maize); WO 91/10725 (maize); Klein, et al., (1988) *Plant Physiol.* 91:440-444 (maize); Fromm, et al., (1990) *Biotechnology* 8:833-839 and Gordon-Kamm, et al., (1990) *Plant Cell* 2:603-618 (maize); Hooydaas-Van Slogteren and Hooykaas (1984) *Nature* (London) 311:763-764; Bytebierm, et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet, et al., (1985) *In The Experimental Manipulation of Ovule Tissues, ed. Chapman, et al., pp.* 197-209, Longman, N.Y. (pollen); Kaeppler, et al., (1990) *Plant Cell Reports* 9:415-418 and Kaeppler, et al., (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); U.S. Pat. No. 5,693,512 (sonication); D'Halluin, et al., (1992) *Plant Cell* 4:1495-1505 (electroporation); Li, et al., (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford, (1995) *Annals of Botany* 75:407-413 (rice); Osjoda, et al., (1996) *Nature Biotech.* 14:745-750; *Agrobacterium* mediated maize transformation (U.S. Pat. No. 5,981,840); silicon carbide whisker methods (Frame, et al., (1994) *Plant J.* 6:941-948); laser methods (Guo, et al., (1995) *Physiologia Plantarum* 93:19-24); sonication methods (Bao, et al., (1997) *Ultrasound in Medicine & Biology* 23:953-959; Finer and Finer, (2000) *Lett Appl Microbiol.* 30:406-10; Amoah, et al., (2001) *J Exp Bot* 52:1135-42); polyethylene glycol methods (Krens, et al., (1982) *Nature* 296:72-77); protoplasts of monocot and dicot cells can be transformed using electroporation (Fromm, et al., (1985) *Proc. Natl. Acad. Sci. USA* 82:5824-5828) and microinjection (Crossway, et al., (1986) *Mol. Gen. Genet.* 202:179-185), all of which are herein incorporated by reference.

Agrobacterium-Mediated Transformation

[0141] A widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium. A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of plants. See, e.g., Kado, (1991) *Crit. Rev. Plant Sci.* 10:1. Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber, et al., supra; Miki, et al., supra and Moloney, et al., (1989) *Plant Cell Reports* 8:238.

[0142] Similarly, a polynucleotide of interest can be inserted into the T-DNA region of a Ti or Ri plasmid derived from *A. tumefaciens* or *A. rhizogenes*, respectively. Thus, expression cassettes can be constructed as above, using these plasmids. Many control sequences are known which when coupled to a heterologous coding sequence and transformed into a host organism show fidelity in gene expression with respect to tissue/organ specificity of the original coding sequence. See, e.g., Benfey and Chua, (1989) *Science* 244: 174-81. Particularly suitable control sequences for use in these plasmids are promoters for constitutive expression of the gene in the various target plants. Other useful control sequences include a promoter and terminator from the nopaline synthase gene (NOS). The NOS promoter and terminator are present in the plasmid pARC2, available from the American Type Culture Collection and designated ATCC 67238. If such a system is used, the virulence (vir) gene from either the Ti or Ri plasmid must also be present, either along with the T-DNA portion or via a binary system where the vir gene is present on a separate vector. Such systems, vectors for use therein, and methods of transforming plant cells are described in U.S. Pat. No. 4,658,082; US Patent Application Serial Number 913,914, filed Oct. 1, 1986, as referenced in U.S. Pat. No. 5,262,306, issued Nov. 16, 1993 and Simpson, et al., (1986) *Plant Mol. Biol.* 6:403-15 (also referenced in the '306 patent), all incorporated by reference in their entirety.

[0143] Once constructed, these plasmids can be placed into *A. rhizogenes* or *A. tumefaciens* and these vectors used to transform cells of plant species, including but not limited to soybean, maize, sorghum, alfalfa, rice, clover, cabbage, banana, coffee, celery, tobacco, cowpea, cotton, melon and pepper. The selection of either *A. tumefaciens* or *A. rhizogenes* will depend on the plant being transformed thereby. In general *A. tumefaciens* is the preferred organism for transformation. Most dicotyledonous plants, some gymnosperms and a few monocotyledonous plants (e.g., certain members of the Liliales and Arales) are susceptible to infection with *A. tumefaciens. A. rhizogenes* also has a wide host range, embracing most dicots and some gymnosperms, which includes members of the *Leguminosae, Compositae* and *Chenopodiaceae*. Monocot plants can now be transformed with some success. EP Patent Number 604662 B1 discloses a method for transforming monocots using *Agrobacterium*. EP Patent Number 672752 B1 discloses a method for transforming monocots with *Agrobacterium* using the scutellum of immature embryos. Ishida, et al., discuss a method for transforming maize by exposing immature embryos to *A. tumefaciens* (*Nature Biotechnology* 14:745-50 (1996)).

[0144] Once transformed, these cells can be used to regenerate transgenic plants. For example, whole plants can be infected with these vectors by wounding the plant and then introducing the vector into the wound site. Any part of the plant can be wounded, including leaves, stems and roots. Roots or shoots transformed by inoculation of plant tissue with *A. rhizogenes* or *A. tumefaciens* can be used as a source of plant tissue to regenerate transgenic plants, either via somatic embryogenesis or organogenesis. Alternatively, plant tissue, in the form of an explant, such as cotyledonary tissue or leaf disks, can be inoculated with these vectors and cultured under conditions which promote plant regeneration. Examples of such methods for regenerating plant tissue are known to those of skill in the art.

Direct Gene Transfer

[0145] Despite the fact that the host range for *Agrobacterium*-mediated transformation is broad, some major cereal crop species and gymnosperms were initially recalcitrant to this mode of gene transfer. Success and refinements have been reported, both for *Agrobacterium*-mediated transformation and for alternative methods, collectively referred to as direct gene transfer. For example, with respect to rice, see, Kathuria, et al., (2007) *Critical Reviews in Plant Sciences* 26:65-103. With respect to wheat, see, He, (2010) *J. Exp. Bot* 61(6):1567-1581; XiuDao, et al., (2010) *Sci. Agri. Sinica* 43(8):1539-1553; Zale, (2009) *Plant Cell Rep.* 28(6):903-913; Wang, et al., (2009) *Cereal Res. Commun.* 37(1):1-12; Greer, (2009) *New Biotech.* 26 (½):44-52. With respect to sugar cane, see, van der Vyver, (2010) *Sugar Tech.* 12(1):21-25; Joyce, et al., (2010) *Plant Cell Rep.* 29(2):173-183; Kalunke, et al., (2009) *Sugar Tech.* 11(4):365-369; Gilbert, et al., (2009) *Field Crops Res.* 111 (1-2):39-46. With respect to turfgrass, see, Cao, (2006) *Plant Cell, Tissue, Organ Culture* 85(3):307-316.

[0146] A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4 μm. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes (Sanford, et al., (1987) *Part. Sci. Technol.* 5:27; Sanford, (1988) *Trends Biotech* 6:299; Sanford, (1990) *Physiol. Plant* 79:206 and Klein, et al., (1992) *Biotechnology* 10:268).

[0147] Another method for physical delivery of DNA to plants is sonication of target cells as described in Zang, et al., (1991) *BioTechnology* 9:996. Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, e.g., Deshayes, et al., (1985) *EMBO J.* 4:2731 and Christou, et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:3962. Direct uptake of DNA into protoplasts using $CaCl_2$ precipitation, polyvinyl alcohol or poly-L-ornithine has also been reported. See, e.g., Hain, et al., (1985) *Mol. Gen. Genet.* 199:161 and Draper, et al., (1982) *Plant Cell Physiol.* 23:451.

[0148] Electroporation of protoplasts and whole cells and tissues has also been described. See, e.g., Donn, et al., (1990) *Abstracts of the VIIth Intl. Congress on Plant Cell and Tissue Culture IAPTC*, A2-38, p. 53; D'Halluin, et al., (1992) *Plant Cell* 4:1495-505 and Spencer, et al., (1994) *Plant Mol. Biol.* 24:51-61.

Reducing the Activity and/or Level of an ACS Polypeptide

[0149] Methods are provided to reduce or eliminate the level or activity of an ACS polypeptide by transforming a plant cell with an expression cassette that expresses a polynucleotide that reduces the expression of the ACS polypeptide. The polynucleotide may reduce the expression of the ACS polypeptide directly, by preventing transcription or translation of the ACS messenger RNA, or indirectly, by encoding a polypeptide that reduces the transcription or translation of an ACS gene encoding an ACS polypeptide. Methods for reducing or eliminating the expression of a gene in a plant are well known in the art and any such method may be used in the present invention to reduce the expression of ACS polypeptide.

[0150] The expression of an ACS polypeptide is reduced if the level of the ACS polypeptide is less than 100%, 99% 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% or 1% of the level of the same ACS polypeptide in a control plant. In particular embodiments, the level of the ACS polypeptide in a modified plant is less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5% or less than 2% of the level of the same or a related ACS polypeptide in a control plant. The ACS polynucleotide expression level and/or polypeptide level and/or enzymatic activity may be reduced such that the reduction is phenotypically sufficient to provide tolerance to drought conditions without a yield penalty occurring under well-watered conditions. The level or activity of one or more ACS polynucleotides, polypeptides or enzymes may be impacted. The expression level of the ACS polypeptide may be measured directly, for example, by assaying for the quantity of ACS polypeptide expressed in the plant cell or plant, or indirectly, for example, by measuring the ACS or ethylene synthesis activity in the plant cell or plant or by measuring the phenotypic changes in the plant. Methods for performing such assays are described elsewhere herein.

[0151] In certain embodiments of the invention, the activity of the ACS polypeptide is reduced or eliminated by transforming a plant cell with an expression cassette comprising a polynucleotide encoding a polypeptide that inhibits the activity of an ACS polypeptide. The activity of an ACS polypeptide is reduced if the activity of the ACS polypeptide is less than 100%, 99% 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% or 1% of the activity of the same ACS polypeptide in a control plant. In particular embodiments, the ACS activity of the ACS polypeptide in a modified plant is less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10% or less than 5% of the ACS activity of the same polypeptide in a control plant. The ACS activity of an ACS polypeptide is "eliminated" according to the invention when it is not detectable by the assay methods described elsewhere herein. Methods of determining the alteration of activity of an ACS polypeptide are described elsewhere herein.

[0152] In other embodiments, the activity of an ACS polypeptide may be reduced or eliminated by disrupting or excising at least a part of the gene encoding the ACS polypeptide. Mutagenized plants that carry mutations in ACS genes also result in reduced expression of the ACS gene and/or reduced activity of the encoded ACS polypeptide.

[0153] Thus, many methods may be used to reduce or eliminate the activity of an ACS polypeptide. One or more methods may be used to reduce the activity of a single ACS polypeptide. One or more methods may be used to reduce the activity of multiple ACS polypeptides.

1. Polynucleotide-Based Methods:

[0154] In some embodiments, a plant is transformed with an expression cassette that is capable of expressing a poly-

nucleotide that reduces the expression of an ACS polypeptide. The term "expression" as used herein refers to the biosynthesis of a gene product, including the transcription and/or translation of said gene product. For example, an expression cassette capable of expressing a polynucleotide that reduces the expression of at least one ACS polypeptide is an expression cassette capable of producing an RNA molecule that inhibits the transcription and/or translation of at least one ACS polypeptide. The "expression" or "production" of a protein or polypeptide from a DNA molecule refers to the transcription and translation of the coding sequence to produce the protein or polypeptide, while the "expression" or "production" of a protein or polypeptide from an RNA molecule refers to the translation of the RNA coding sequence to produce the protein or polypeptide.

[0155] Examples of polynucleotides that modulate the expression of an ACS polypeptide are given below.

i. Sense Suppression/Cosuppression

[0156] In some embodiments, down-regulation of the expression of an ACS polypeptide may be accomplished by sense suppression or cosuppression. For cosuppression, an expression cassette is designed to express an RNA molecule corresponding to all or part of a messenger RNA encoding an ACS polypeptide in the "sense" orientation. Over-expression of the RNA molecule can result in reduced expression of the native gene. Accordingly, multiple plant lines transformed with the cosuppression expression cassette are screened to identify those that show the reduction of ACS polypeptide expression.

[0157] The polynucleotide used for cosuppression may correspond to all or part of the sequence encoding the ACS polypeptide, all or part of the 5' and/or 3' untranslated region of an ACS polypeptide transcript or all or part of both the coding sequence and the untranslated regions of a transcript encoding an ACS polypeptide. In some embodiments where the polynucleotide comprises all or part of the coding region for the ACS polypeptide, the expression cassette is designed to eliminate the start codon of the polynucleotide so that no protein product will be translated.

[0158] Cosuppression may be used to inhibit the expression of plant genes to produce plants having undetectable protein levels for the proteins encoded by these genes. See, for example, Broin, et al., (2002) *Plant Cell* 14:1417-1432. Cosuppression may also be used to inhibit the expression of multiple proteins in the same plant. See, for example, U.S. Pat. No. 5,942,657. Methods for using cosuppression to inhibit the expression of endogenous genes in plants are described in Flavell, et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:3490-3496; Jorgensen, et al., (1996) *Plant Mol. Biol.* 31:957-973; Johansen and Carrington, (2001) *Plant Physiol.* 126:930-938; Broin, et al., (2002) *Plant Cell* 14:1417-1432; Stoutjesdijk, et al., (2002) *Plant Physiol.* 129:1723-1731; Yu, et al., (2003) *Phytochemistry* 63:753-763 and U.S. Pat. Nos. 5,034,323, 5,283,184 and 5,942,657, each of which is herein incorporated by reference. The efficiency of cosuppression may be increased by including a poly-dT region in the expression cassette at a position 3' to the sense sequence and 5' of the polyadenylation signal. See, US Patent Application Publication Number 2002/0048814, herein incorporated by reference. Typically, such a nucleotide sequence has substantial sequence identity to the full-length sequence or a fragment or portion of the transcript of the endogenous gene, generally greater than about 65% sequence identity, often greater than about 85% sequence identity, sometimes greater than about 95% sequence identity. See, U.S. Pat. Nos. 5,283,184 and 5,034,323, herein incorporated by reference.

ii. Antisense Suppression

[0159] In some embodiments, reduction of the expression of the ACS polypeptide may be obtained by antisense suppression. For antisense suppression, the expression cassette is designed to express an RNA molecule complementary to all or part of a messenger RNA encoding the ACS polypeptide. Over expression of the antisense RNA molecule can result in reduced expression of the native gene. Accordingly, multiple plant lines transformed with the antisense suppression expression cassette are screened to identify those that show the optimum down-regulation of ACS polypeptide expression.

[0160] The polynucleotide for use in antisense suppression may correspond to all or part of the complement of the sequence encoding the ACS polypeptide, all or part of the complement of the 5' and/or 3' untranslated region of the ACS transcript or all or part of the complement of both the coding sequence and the untranslated regions of a transcript encoding the ACS polypeptide. In addition, the antisense polynucleotide may be fully complementary (i.e., 100% identical to the complement of the target sequence) or partially complementary (i.e., less than 100% identical to the complement of the target sequence) to the target sequence. Antisense suppression may be used to inhibit the expression of multiple proteins in the same plant. See, for example, U.S. Pat. No. 5,942,657. Furthermore, portions of the antisense nucleotides may be used to disrupt the expression of the target gene. Generally, sequences of at least 50 nucleotides, 100 nucleotides, 200 nucleotides, 300, 400, 450, 500, 550 or more nucleotides may be used. Methods for using antisense suppression to inhibit the expression of endogenous genes in plants are described, for example, in Liu, et al., (2002) *Plant Physiol.* 129:1732-1743 and U.S. Pat. Nos. 5,759,829 and 5,942,657, each of which is herein incorporated by reference. Efficiency of antisense suppression may be increased by including a poly-dT region in the expression cassette at a position 3' to the antisense sequence and 5' of the polyadenylation signal. See, US Patent Application Publication Number 2002/0048814, herein incorporated by reference.

iii. Double-Stranded RNA Interference

[0161] In some embodiments of the invention, down-regulation of the expression of an ACS polypeptide may be obtained by double-stranded RNA (dsRNA) interference. For dsRNA interference, a sense RNA molecule like that described above for cosuppression and an antisense RNA molecule that is fully or partially complementary to the sense RNA molecule are expressed in the same cell, resulting in down-regulation of the expression of the corresponding endogenous messenger RNA.

[0162] Expression of the sense and antisense molecules can be accomplished by designing the expression cassette to comprise both a sense sequence and an antisense sequence. Alternatively, separate expression cassettes may be used for the sense and antisense sequences. Multiple plant lines transformed with the dsRNA interference expression cassette or expression cassettes are then screened to identify plant lines that show the optimum down-regulation of ACS polypeptide expression. Methods for using dsRNA interference to inhibit the expression of endogenous plant genes are described in Waterhouse, et al., (1998) *Proc. Natl. Acad. Sci. USA* 95:13959-13964, Liu, et al., (2002) *Plant Physiol.* 129:1732-

1743 and WO 99/49029, WO 99/53050, WO 99/61631 and WO 00/49035, each of which is herein incorporated by reference.

iv. Hairpin RNA Interference and Intron-Containing Hairpin RNA Interference

[0163] In some embodiments of the invention, down-regulation of the expression of an ACS polypeptide may be obtained by hairpin RNA (hpRNA) interference or intron-containing hairpin RNA (ihpRNA) interference. These methods are highly efficient at inhibiting the expression of endogenous genes. See, Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38 and the references cited therein.

[0164] For hpRNA interference, the expression cassette is designed to express an RNA molecule that hybridizes with itself to form a hairpin structure that comprises a single-stranded loop region and a base-paired stem. The base-paired stem region comprises a sense sequence corresponding to all or part of the endogenous messenger RNA encoding the gene whose expression is to be inhibited and an antisense sequence that is fully or partially complementary to the sense sequence. The antisense sequence may be located "upstream" of the sense sequence (i.e., the antisense sequence may be closer to the promoter driving expression of the hpRNA than is the sense sequence.) The base-paired stem region may correspond to a portion of a promoter sequence controlling expression of the gene to be inhibited. Thus, the base-paired stem region of the molecule generally determines the specificity of the RNA interference. The sense sequence and the antisense sequence are generally of similar lengths but may differ in length. Thus, these sequences may be portions or fragments of at least 10, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 70, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 500, 600, 700, 800 or 900 nucleotides in length or at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 kb in length. The loop region of the expression cassette may vary in length. Thus, the loop region may be at least 50, 80, 100, 200, 300, 400, 500, 600, 700, 800 or 900 nucleotides in length or at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 kb in length.

[0165] hpRNA molecules are highly efficient at inhibiting the expression of endogenous genes and the RNA interference they induce is inherited by subsequent generations of plants. See, for example, Chuang and Meyerowitz, (2000) *Proc. Natl. Acad. Sci. USA* 97:4985-4990; Stoutjesdijk, et al., (2002) *Plant Physiol.* 129:1723-1731 and Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38. Methods for using hpRNA interference to reduce or silence the expression of genes are described, for example, in Chuang and Meyerowitz, (2000) *Proc. Natl. Acad. Sci. USA* 97:4985-4990; Stoutjesdijk, et al., (2002) *Plant Physiol.* 129:1723-1731; Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38; Pandolfini et al., *BMC Biotechnology* 3:7 and US Patent Application Publication Number 2003/0175965, each of which is herein incorporated by reference. A transient assay for the efficiency of hpRNA constructs to silence gene expression in vivo has been described by Panstruga, et al., (2003) *Mol. Biol. Rep.* 30:135-140, herein incorporated by reference.

[0166] For ihpRNA, the interfering molecules have the same general structure as for hpRNA, but the RNA molecule additionally comprises an intron that is capable of being spliced in the cell in which the ihpRNA is expressed. The use of an intron minimizes the size of the loop in the hairpin RNA molecule following splicing and this increases the efficiency of interference. In some embodiments, the intron is the Adh1 intron 1. Methods for using ihpRNA interference to inhibit the expression of endogenous plant genes are described, for example, in Smith, et al., (2000) *Nature* 407:319-320. In fact, Smith, et al., show 100% suppression of endogenous gene expression using ihpRNA-mediated interference. Methods for using ihpRNA interference to inhibit the expression of endogenous plant genes are described, for example, in Smith, et al., (2000) *Nature* 407:319-320; Wesley, et al., (2001) *Plant J.* 27:581-590; Wang and Waterhouse, (2001) *Curr. Opin. Plant Biol.* 5:146-150; Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38; Helliwell and Waterhouse, (2003) *Methods* 30:289-295 and US Patent Application Publication Number 2003/0180945, each of which is herein incorporated by reference.

[0167] The expression cassette for hpRNA interference may also be designed such that the sense sequence and the antisense sequence do not correspond to an endogenous is RNA. In this embodiment, the sense and antisense sequence flank a loop sequence that comprises a nucleotide sequence corresponding to all or part of the endogenous messenger RNA of the target gene. Thus, it is the loop region that determines the specificity of the RNA interference. See, for example, WO 02/00904; Mette, et al., (2000) *EMBO J.* 19:5194-5201; Matzke, et al., (2001) *Curr. Opin. Genet. Devel.* 11:221-227; Scheid, et al., (2002) *Proc. Natl. Acad. Sci., USA* 99:13659-13662; Aufsaftz, et al., (2002) *Proc. Natl. Acad. Sci.* 99(4):16499-16506; Sijen, et al., Curr. Biol. (2001) 11:436-440), herein incorporated by reference.

v. Amplicon-Mediated Interference

[0168] Amplicon expression cassettes comprise a plant-virus-derived sequence that contains all or part of the target gene but generally not all of the genes of the native virus. The viral sequences present in the transcription product of the expression cassette allow the transcription product to direct its own replication. The transcripts produced by the amplicon may be either sense or antisense relative to the target sequence (i.e., the messenger RNA for the ACS polypeptide). Methods of using amplicons to inhibit the expression of endogenous plant genes are described, for example, in Angell and Baulcombe, (1997) *EMBO J.* 16:3675-3684, Angell and Baulcombe, (1999) *Plant J.* 20:357-362 and U.S. Pat. No. 6,635,805, each of which is herein incorporated by reference.

vi. Ribozymes

[0169] In some embodiments, the polynucleotide expressed by the expression cassette is catalytic RNA or has ribozyme activity specific for the messenger RNA of the ACS polypeptide. Thus, the polynucleotide causes the degradation of the endogenous messenger RNA, resulting in reduced expression of the ACS polypeptide. This method is described, for example, in U.S. Pat. No. 4,987,071, herein incorporated by reference.

Methods for Modulating Drought Tolerance in a Plant

[0170] Methods for modulating drought tolerance in plants are also features of the invention. The ability to introduce different degrees of drought tolerance into plants offers flexibility in the use of the invention: for example, introduction of strong drought tolerance for improved grain-filling or for silage in areas with longer or drier growing seasons, versus the introduction of a moderate drought tolerance for silage in agricultural areas with shorter growing seasons. Modulation of drought tolerance of a plant of the invention may reflect one or more of the following: (a) a reduction in the production of at least one ACC-synthase-encoding mRNA; (b) a reduction

in the production of an ACC synthase; (c) a reduction in the production of ACC; (d) a reduction in the production of ethylene; (e) an increase in plant height or (f) any combination of (a)-(e), compared to a corresponding control plant.

[0171] For example, a method of the invention can include: (a) selecting at least one ACC synthase gene to mutate, thereby providing at least one desired ACC synthase gene; (b) introducing a mutant form of the at least one desired ACC synthase gene into the plant and (c) expressing the mutant form, thereby modulating drought tolerance in the plant. Plants produced by such methods are also a feature of the invention.

[0172] The degree of drought tolerance introduced into a plant can be determined by a number of factors, e.g., which ACC synthase gene is selected, whether the mutant gene member is present in a heterozygous or homozygous state or by the number of members of this family which are inactivated or by a combination of two or more such factors.

[0173] Once the desired ACC synthase gene is selected, a mutant form of the ACC synthase gene is introduced into a plant. In certain embodiments, the mutant form is introduced by *Agrobacterium*-mediated transfer, electroporation, microprojectile bombardment, homologous recombination or a sexual cross. In certain embodiments, the mutant form includes, e.g., a heterozygous mutation in the at least one ACC synthase gene, a homozygous mutation in the at least one ACC synthase gene or a combination of homozygous mutation and heterozygous mutation if more than one ACC synthase gene is selected. In another embodiment, the mutant form includes a subsequence of the at least one desired ACC synthase gene in an antisense, sense or RNA silencing or interference configuration.

[0174] Expression of the mutant form of the ACC synthase gene can be determined in a number of ways. For example, detection of expression products is performed either qualitatively (presence or absence of one or more product of interest) or quantitatively (by monitoring the level of expression of one or more product of interest). In one embodiment, the expression product is an RNA expression product. The invention optionally includes monitoring an expression level of a nucleic acid or polypeptide as noted herein for detection of ACC synthase in a plant or in a population of plants. Monitoring levels of ethylene or ACC can also serve to detect down-regulation of expression or activity of the ACC synthase gene.

Methods for Modulating Density Tolerance in a Plant

[0175] In addition to increasing tolerance to drought stress in plants of the invention compared to a control plant, the invention also enables higher density planting of plants of the invention, leading to increased yield per acre of corn. Most of the increased yield per acre of corn over the last century has come from increasing tolerance to density, which is a stress to plants. Methods for modulating plant stress response, e.g., increasing tolerance for density, are also a feature of the invention. For example, a method of the invention can include: (a) selecting at least one ACC synthase gene to mutate, thereby providing at least one desired ACC synthase gene; (b) introducing a mutant form of the at least one desired ACC synthase gene into the plant and (c) expressing the mutant form, thereby modulating density tolerance in the plant. Plants produced by such methods are also a feature of the invention. When ethylene production is reduced in a plant by a mutant form of a desired ACC synthase gene, the plant may have a reduced perception of and/or response to density. Thus, plants of the invention can be planted at higher density than currently practiced by farmers and produce an increase in yield of seed and/or biomass.

Methods for Modulating Nitrogen Utilization Efficiency in a Plant

[0176] In addition to increasing tolerance to drought stress and improving density stress tolerance in plants of the invention compared to a control plant, the invention also may provide greater nitrogen utilization efficiency. For example, a method of the invention can include: (a) selecting at least one ACC synthase gene to mutate, thereby providing at least one desired ACC synthase gene; (b) introducing a mutant form of the at least one desired ACC synthase gene into the plant and (c) expressing the mutant form, thereby modulating NUE in the plant. Plants produced by such methods are also a feature of the invention. Plants in which NUE is improved may be more productive than control plants under comparable conditions of ample nitrogen availability and/or may maintain productivity under significantly reduced nitrogen availability. Improved NUE may be reflected in one or more attributes such as increased biomass, increased grain yield, increased harvest index, increased photosynthetic rates and increased tolerance to biotic or abiotic stress. In particular, improving NUE in maize would increase harvestable yield per unit of input nitrogen fertilizer, both in developing nations where access to nitrogen fertilizer is limited and in developed nations where the level of nitrogen use remains high.

Screening/Characterization of Plants or Plant Cells

[0177] Plants can be screened and/or characterized genotypically, biochemically, phenotypically or by a combination of two or more of these methods. For example, plants may be characterized to determine the presence, absence and/or expression level (e.g., amount, modulation, such as a decrease or increase compared to a control cell) of a polynucleotide of the invention; the presence, absence, expression and/or enzymatic activity of a polypeptide of the invention and/or modulation of drought tolerance, modulation of nitrogen use efficiency, modulation of density tolerance and/or modulation of ethylene production.

[0178] Chemicals, e.g., ethylene, ACC, etc., can be recovered and assayed from the cell extracts. For example, internal concentrations of ACC can be assayed by gas chromatography-mass spectroscopy, in acidic plant extracts as ethylene after decomposition in alkaline hypochlorite solution, etc. The concentration of ethylene can be determined by, e.g., gas chromatography-mass spectroscopy, etc. See, e.g., Nagahama, et al., (1991) *J. Gen. Microbiol.* 137:2281 2286. For example, ethylene can be measured with a gas chromatograph equipped with, e.g., an alumina based column (such as an HP-PLOT A1203 capillary column (Agilent Technologies, Santa Clara, Calif.) and a flame ionization detector.

[0179] Phenotypic analysis includes, e.g., analyzing changes in chemical composition, morphology or physiological properties of the plant. For example, phenotypic changes can include, but are not limited to, an increase in drought tolerance, an increase in density tolerance, an increase in nitrogen use efficiency and a decrease in ethylene production.

[0180] A variety of assays can be used for monitoring drought tolerance and/or NUE. For example, assays include, but are not limited to, visual inspection, monitoring photo-

synthesis measurements and measuring levels of chlorophyll, DNA, RNA and/or protein content of, e.g., the leaves, under stress and non-stress conditions.

Plants of the Invention

[0181] Plant cells useful in the invention include, but are not limited to, meristem cells, Type I, Type II and Type III callus, immature embryos and gametic cells such as microspores, pollen, sperm and egg. In certain embodiments, the plant cell of the invention is from a dicot or monocot. A plant regenerated from the plant cell(s) of the invention is also a feature of the invention.

[0182] In one embodiment, the plant cell is in a plant, e.g., a hybrid plant, comprising a drought tolerant phenotype. In another embodiment, the plant cell is in a plant comprising a sterility phenotype, e.g., a male sterility phenotype. Through a series of breeding manipulations, the construct impacting an ACC synthase gene can be moved from one plant line to another plant line. For example, a hybrid plant can be produced by sexual cross of a plant comprising a modified expression of one or more ACC synthase genes and a control plant.

[0183] Modified plant cells are also a feature of the invention. In a first aspect, the invention provides for an isolated or recombinant plant cell comprising at least one down-regulation construct capable of inhibiting an endogenous ACC synthase gene; e.g., a nucleic acid sequence, or complement thereof, comprising, e.g., at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 99.5% or more, sequence identity to the ACS6 down-regulation expression construct of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. The down-regulation of expression or activity of at least one ACC synthase polynucleotide or protein is compared to a corresponding control plant cell lacking the down-regulation construct. Essentially any plant can be used in the methods and compositions of the invention. Such species include, but are not restricted to, members of the families Poaceae (formerly Graminae), including *Zea mays* (corn or maize), rye, triticale, barley, millet, rice, wheat, oats, etc.; Leguminosae, including pea, beans, lentil, peanut, yam bean, cowpeas, velvet beans, soybean, clover, alfalfa, lupine, vetch, lotus, sweet clover, wisteria, sweetpea, etc.; Compositae, the largest family of vascular plants, including at least 1,000 genera, including important commercial crops such as sunflower; Rosaciae, including raspberry, apricot, almond, peach, rose, etc.; as well as nut plants, including, walnut, pecan, hazelnut, etc., forest trees (including *Pinus, Quercus, Pseutotsuga, Sequoia, Populus*, etc. and other common crop plants, e.g., cotton, sorghum, lawn grasses, tomato, potato, pepper, canola, broccoli, cabbage, etc.

[0184] Additional plants, as well as those specified above, include plants from the genera: *Acamptoclados, Achnatherum, Achnella, Acroceras, Aegilops, Aegopgon, Agroelymus, Agrohordeum, Agropogon, Agropyron, Agrositanion, Agrostis, Aira, Allolepis, Alloteropsis, Alopecurus, Amblyopyrum, Ammophila, Ampelodesmos, Amphibromus, Amphicarpum, Amphilophis, Anastrophus, Anatherum, Andropogron, Anemathele, Aneurolepidium, Anisantha, Anthaenantia, Anthephora, Anthochloa, Anthoxanthum, Apera, Apluda, Archtagrostis, Arctophila, Argillochloa, Aristida, Arrhenatherum, Arthraxon, Arthrostylidium, Arundinaria, Arundinella, Arundo, Aspris, Atheropogon, Avena* (e.g., oats), *Avenella, Avenochloa, Avenula, Axonopus, Bambusa, Beckmannia, Blepharidachne, Blepharoneuron, Bothriochloa, Bouteloua, Brachiaria, Brachyelytrum, Brachypodium, Briza, Brizopyrum, Bromelica, Bromopsis, Bromus, Buchloe, Bulbilis, Calamagrostis, Calamovilfa, Campulosus, Capriola, Catabrosa, Catapodium, Cathestecum, Cenchropsis, Cenchrus, Centotheca, Ceratochloa, Chaetochloa, Chasmanthium, Chimonobambusa, Chionochloa, Chloris, Chondrosum, Chrysopon, Chusquea, Cinna, Cladoraphis, Coelorachis, Coix, Coleanthus, Colpodium, Coridochloa, Cornucopiae, Cortaderia, Corynephorus, Cottea, Critesion, Crypsis, Ctenium, Cutandia, Cylindropyrum, Cymbopogon, Cynodon, Cynosurus, Cytrococcum, Dactylis, Dactyloctenium, Danthonia, Dasyochloa, Dasyprum, Davyella, Dendrocalamus, Deschampsia, Desmazeria, Deyeuxia, Diarina, Diarrhena, Dichanthelium, Dichanthium, Dichelachne, Diectomus, Digitaria, Dimeria, Dimorpostachys, Dinebra, Diplachne, Dissanthelium, Dissochondrus, Distichlis, Drepanostachyum, Dupoa, Dupontia, Echinochloa, Ectosperma, Ehrharta, Eleusine, Elyhordeum, Elyleymus, Elymordeum, Elymus, Elyonurus, Elysitanion, Elytesion, Elytrigia, Enneapogon, Enteropogon, Epicampes, Eragrostis, Eremochloa, Eremopoa, Eremopyrum, Erianthus, Ericoma, Erichloa, Eriochrysis, Erioneuron, Euchlaena, Euclasta, Eulalia, Eulaliopsis, Eustachys, Fargesia, Festuca, Festulolium, Fingerhuthia, Fluminia, Garnotia, Gastridium, Gaudinia, Gigantochloa, Glyceria, Graphephorum, Gymnopogon, Gynerium, Hackelochloa, Hainardia, Hakonechloa, Haynaldia, Heleochloa, Helictotrichon, Hemarthria, Hesperochloa, Hesperostipa, Heteropogon, Hibanobambusa, Hierochloe, Hilaria, Holcus, Homalocenchrus, Hordeum* (e.g., barley), *Hydrochloa, Hymenachne, Hyparrhenia, Hypogynium, Hystrix, Ichnanthus, Imperata, Indocalamus, Isachne, Ischaemum, Ixophorus, Koeleria, Korycarpus, Lagurus, Lamarckia, Lasiacis, Leersia, Leptochloa, Leptochloopsis, Leptocoryphium, Leptoloma, Leptogon, Lepturus, Lerchenfeldia, Leucopoa, Leymostachys, Leymus, Limnodea, Lithachne, Lolium, Lophochlaena, Lophochloa, Lophopyrum, Ludolfia, Luziola, Lycurus, Lygeum, Maltea, Manisuris, Megastachya, Melica, Melinis, Mibora, Microchloa, Microlaena, Microstegium, Milium, Miscanthus, Mnesithea, Molinia, Monanthochloe, Monerma, Monroa, Muhlenbergia, Nardus, Nassella, Nazia, Neeragrostis, Neoschischkinia, Neostapfia, Neyraudia, Nothoholcus, Olyra, Opizia, Oplismenus, Orcuttia, Oryza* (e.g., rice), *Oryzopsis, Otatea, Oxytenanthera, Particularia, Panicum, Pappophorum, Parapholis, Pascopyrum, Paspalidium, Paspalum, Pennisetum* (e.g., millet), *Phalaris, Phalaroides, Phanopyrum, Pharus, Phippsia, Phleum, Pholiurus, Phragmites, Phyllostachys, Piptatherum, Piptochaetium, Pleioblastus, Pleopogon, Pleuraphis, Pleuropogon, Poa, Podagrostis, Polypogon, Polytrias, Psathyrostachys, Pseudelymus, Pseudoroegneria, Pseudosasa, Ptilagrostis, Puccinellia, Pucciphippsia, Redfieldia, Reimaria, Reimarochloa, Rhaphis, Rhombolytrum, Rhynchelytrum, Roegneria, Rostraria, Rottboellia, Rytilix, Saccharum, Sacciolepis, Sasa, Sasaella, Sasamorpha, Savastana, Schedonnardus, Schismus, Schizachne, Schizachyrium, Schizostachyum, Sclerochloa, Scleropoa, Scleropogon, Scolochloa, Scribneria, Secale* (e.g., rye), *Semiarundinaria, Sesleria, Setaria, Shibataea, Sieglingia, Sinarundinaria, Sinobambusa, Sinocalamus, Sitanion, Sorghastrum, Sorghum, Spartina, Sphenopholis, Spodiopogon, Sporobolus, Stapfia, Steinchisma, Stenotaphrum, Stipa, Stipagrostis, Stiporyzopsis, Swallenia, Syntherisma, Taeniatherum, Terrellia, Terrelymus, Thamno-*

calamus, Themeda, Thinopyrum, Thuarea, Thysanolaena, Torresia, Torreyochloa, Trachynia, Trachypogon, Tragus, Trichachne, Trichloris, Tricholaena, Trichoneura, Tridens, Triodia, Triplasis, Tripogon, Tripsacum, Trisetobromus, Trisetum, Triticosecale, Triticum (e.g., wheat), *Tuctoria, Uniola, Urachne, Uralepis, Urochloa, Vahlodea, Valota, Vaseyochloa, Ventenata, Vetiveria, Vilfa, Vulpia, Willkommia, Yushania, Zea* (e.g., corn), *Zizania, Zizaniopsis and Zoysia.*

Regeneration of Isolated, Recombinant or Transgenic Plants

[0185] Transformed plant cells which are derived by plant transformation techniques and isolated or recombinant plant cells derived therefrom, including those discussed above, can be cultured to regenerate a whole plant which possesses the desired genotype (i.e., comprising an ACC synthase down-regulation nucleic acid) and/or thus the desired phenotype, e.g., improved NUE and/or drought tolerance phenotype, density tolerant phenotype, etc. The desired cells, which can be identified, e.g., by selection or screening, are cultured in medium that supports regeneration. The cells can then be allowed to mature into plants. For example, such regeneration techniques can rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced into the plant together with the desired nucleotide sequences. Alternatively, cells, tissues or plants can be screened for down-regulation of expression and/or activity of ACC synthase, reduction in ethylene production conferred by the ACC synthase down-regulation nucleic acid sequence, etc. Plant regeneration from cultured protoplasts is described in Evans, et al., (1983) Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp 124 176, Macmillan Publishing Company, New York; Davey, (1983) *Protoplasts*, pp. 12-29, Birkhauser, Basal 1983; Dale, (1983) *Protoplasts* pp. 31-41, Birkhauser, Basel and Binding (1985) *Regeneration of Plants, Plant Protoplasts* pp 21-73, CRC Press, Boca Raton. Regeneration can also be obtained from plant callus, explants, organs or parts thereof. Such regeneration techniques are described generally in Klee, et al., (1987) *Ann Rev of Plant Phys* 38:467-486. See also, e.g., Payne and Gamborg. For transformation and regeneration of maize see, for example, U.S. Pat. No. 5,736,369.

[0186] Plants cells transformed with a plant expression vector can be regenerated, e.g., from single cells, callus tissue or leaf discs according to standard plant tissue culture techniques. It is well known in the art that various cells, tissues and organs from almost any plant can be successfully cultured to regenerate an entire plant. Plant regeneration from cultured protoplasts is described in Evans, et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, Macmillilan Publishing Company, New York, pp. 124-176 (1983) and Binding, Regeneration of Plants, Plant Protoplasts, CRC Press, Boca Raton, pp. 21-73 (1985).

[0187] The regeneration of plants containing the foreign gene introduced by *Agrobacterium* from leaf explants can be achieved as described by Horsch, et al., (1985) *Science* 227: 1229-1231. After transformation with *Agrobacterium*, the explants typically are transferred to selection medium. One of skill will realize that the selection medium depends on the selectable marker that is co-transfected into the explants. In this procedure, transformants are grown in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant species being transformed as described by Fraley, et al., (1983) *Proc. Nat'l. Acad. Sci. USA,* 80:4803. This procedure typically produces shoots, e.g., within two to four weeks, and these transformant shoots (which are typically about 1-2 cm in length) are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Selective pressure is typically maintained in the root and shoot medium.

[0188] Typically, the transformants will develop roots in about 1-2 weeks and form plantlets. After the plantlets are about 3-5 cm in height, they are placed in sterile soil in fiber pots. Those of skill in the art will realize that different acclimation procedures are used to obtain transformed plants of different species. For example, after developing a root and shoot, cuttings, as well as somatic embryos of transformed plants, are transferred to medium for establishment of plantlets. For a description of selection and regeneration of transformed plants, see, e.g., Dodds and Roberts, (1995) Experiments in Plant Tissue Culture, 3rd Ed., Cambridge University Press. Transgenic plants may be fertile or sterile.

[0189] The regeneration of plants from either single plant protoplasts or various explants is well known in the art. See, for example, Methods for Plant Molecular Biology, Weissbach and Weissbach, eds., Academic Press, Inc., San Diego, Calif. (1988). This regeneration and growth process includes the steps of selection of transformant cells and shoots, rooting the transformant shoots and growth of the plantlets in soil. For maize cell culture and regeneration see generally, The Maize Handbook, Freeling and Walbot, Eds., Springer, N.Y. (1994); Corn and Corn Improvement, 3rd edition, Sprague and Dudley, Eds., American Society of Agronomy, Madison, Wis. (1988).

[0190] One of skill will recognize that after the recombinant expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

[0191] In vegetatively propagated crops, mature transgenic plants can be propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants. Selection of desirable transgenics is made and new varieties are obtained and propagated vegetatively for commercial use. In seed-propagated crops, mature transgenic plants can be self-pollinated to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced heterologous nucleic acid. These seeds can be grown to produce plants that would produce the selected phenotype. Mature transgenic plants can also be crossed with other appropriate plants, generally another inbred or hybrid, including, for example, an isogenic untransformed inbred.

[0192] Parts obtained from the regenerated plant, such as flowers, seeds, leaves, branches, fruit and the like are included in the invention, provided that these parts comprise cells comprising the down-regulation construct or a functional fragment thereof. Progeny and variants and mutants of the regenerated plants are also included within the scope of the invention, provided that these plants comprise the down-regulation construct or a functional fragment thereof.

[0193] Transgenic plants expressing the selectable marker can be screened for transmission of the down-regulation construct by, for example, standard immunoblot and DNA detection techniques. Transgenic lines are also typically evaluated for levels of expression of the heterologous nucleic acid. Expression at the RNA level can be determined initially to identify and quantitate expression-positive plants. Standard

techniques for RNA analysis can be employed and include PCR amplification assays using oligonucleotide primers designed to amplify only the heterologous RNA templates and solution hybridization assays using heterologous nucleic acid-specific probes. In addition, in situ hybridization and immunocytochemistry according to standard protocols can be done using heterologous nucleic acid specific polynucleotide probes to localize sites of expression within transgenic tissue. Generally, a number of transgenic lines are screened for the incorporated nucleic acid to identify and select plants with the most appropriate expression profiles.

[0194] Some embodiments comprise a transgenic plant that is homozygous for the added heterologous nucleic acid; i.e., a transgenic plant that contains two added nucleic acid sequences at corresponding loci on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) a heterozygous (aka hemizygous) transgenic plant that contains a single added heterologous nucleic acid, germinating some of the seed produced and analyzing the resulting plants produced for altered expression of a polynucleotide of the present invention relative to a control plant. Back-crossing to a parental plant and out-crossing with a non-transgenic plant or with a plant transgenic for the same or another trait or traits are also contemplated.

[0195] It is also expected that the transformed plants will be used in traditional breeding programs, including TOPCROSS pollination systems as disclosed in U.S. Pat. No. 5,706,603 and U.S. Pat. No. 5,704,160, the disclosure of each of which is incorporated herein by reference.

[0196] In addition to Berger, Ausubel and Sambrook, useful general references for plant cell cloning, culture and regeneration include Jones, (ed) (1995) Plant Gene Transfer and Expression Protocols—Methods in Molecular Biology, Volume 49 Humana Press Towata N.J.; Payne, et al., (1992) Plant Cell and Tissue Culture in Liquid Systems, John Wiley & Sons, Inc. New York, N.Y. (Payne) and Gamborg and Phillips, (eds) (1995) Plant Cell, Tissue and Organ Culture; Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York) (Gamborg). A variety of cell culture media are described in Atlas and Parks, (eds) The Handbook of Microbiological Media (1993) CRC Press, Boca Raton, Fla. (Atlas). Additional information for plant cell culture is found in available commercial literature such as the Life Science Research Cell Culture Catalogue (1998) from Sigma-Aldrich, Inc (St. Louis, Mo.) (Sigma-LSRCCC) and, e.g., the Plant Culture Catalogue and supplement (1997) also from Sigma-Aldrich, Inc (St Louis, Mo.) (Sigma-PCCS). Additional details regarding plant cell culture are found in Croy, (ed.) (1993) Plant Molecular Biology Bios Scientific Publishers, Oxford, UK.

"Stacking" of Constructs and Traits

[0197] In certain embodiments, the nucleic acid sequences of the present invention can be used in combination ("stacked") with other polynucleotide sequences of interest in order to create plants with a desired phenotype. The polynucleotides of the present invention may be stacked with any gene or combination of genes and the combinations generated can include multiple copies of any one or more of the polynucleotides of interest. Stacking can be performed either through molecular stacking or through a conventional breeding approach. Site-specific integration of one or more transgenes at the ACS locus is also possible. The desired combination may affect one or more traits; that is, certain combinations may be created for modulation of gene expression affecting ACC synthase activity and/or ethylene production. Other combinations may be designed to produce plants with a variety of desired traits, including but not limited to traits desirable for animal feed such as high oil genes (e.g., U.S. Pat. No. 6,232,529); balanced amino acids (e.g. hordothionins (U.S. Pat. Nos. 5,990,389; 5,885,801; 5,885,802 and 5,703,409); barley high lysine (Williamson, et al., (1987) *Eur. J. Biochem.* 165:99-106 and WO 98/20122) and high methionine proteins (Pedersen, et al., (1986) *J. Biol. Chem.* 261:6279; Kirihara, et al., (1988) *Gene* 71:359 and Musumura, et al., (1989) *Plant Mol. Biol.* 12:123)); increased digestibility (e.g., modified storage proteins (U.S. patent application Ser. No. 10/053,410, filed Nov. 7, 2001) and thioredoxins (U.S. patent application Ser. No. 10/005,429, filed Dec. 3, 2001)), the disclosures of which are herein incorporated by reference. The polynucleotides of the present invention can also be stacked with traits desirable for insect, disease or herbicide resistance (e.g., *Bacillus thuringiensis* toxic proteins (U.S. Pat. Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; Geiser, et al., (1986) *Gene* 48:109); lectins (Van Damme, et al., (1994) *Plant Mol. Biol.* 24:825); fumonisin detoxification genes (U.S. Pat. No. 5,792,931); avirulence and disease resistance genes (Jones, et al., (1994) *Science* 266:789; Martin, et al., (1993) *Science* 262:1432; Mindrinos, et al., (1994) *Cell* 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations; inhibitors of glutamine synthase such as phosphinothricin or basta (e.g., bar gene) and glyphosate resistance (EPSPS and/or glyphosate N-acetyltransferase (GAT) genes; see, for example, U.S. Pat. Nos. 7,462,481; 7,531,339; 7,405,075; 7,666,644; 7,622,641 and 7,714,188); and traits desirable for processing or process products such as high oil (e.g., U.S. Pat. No. 6,232,529); modified oils (e.g., fatty acid desaturase genes (U.S. Pat. No. 5,952,544; WO 94/11516)); modified starches (e.g., ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (SDBE)) and polymers or bioplastics (e.g., U.S. Pat. No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase and acetoacetyl-CoA reductase (Schubert, et al., (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)), the disclosures of which are herein incorporated by reference. One could also combine the polynucleotides of the present invention with polynucleotides affecting agronomic traits such as male sterility (e.g., see, U.S. Pat. No. 5,583,210), stalk strength, flowering time or transformation technology traits such as cell cycle regulation or gene targeting (e.g. WO 99/61619; WO 00/17364; WO 99/25821), the disclosures of which are herein incorporated by reference.

[0198] For example, in addition to an ACS downregulation expression cassette (which may be an ACS6 downregulation expression cassette), a stacked combination may include one or more expression cassettes providing one or more of the following: modulation of ABA perception/response targeted to reproductive tissues (e.g., eep1 promoter driving *Arabidopsis* ABI1 mutant; see, US Patent Publication Number 2004/0148654); modulation of cytokinin expression or activity (see, e.g., US Patent Publication Number 2009/0165177 and U.S. Pat. No. 6,992,237); modulation of cis-prenyltransferase expression or activity (see, e.g., U.S. Pat. Nos. 6,645,747 and 7,273,737; modulation of cellulose synthase (see,

e.g., U.S. Pat. Nos. 7,214,852 and 7,524,933). In one or more of these stacks, the ACS downregulation expression cassette may comprise a tissue-preferred promoter (see, e.g., the eep5 promoter disclosed in US Patent Publication Number 2009/0307800 or the eep1 promoter disclosed in US Patent Publication Number 2004/0237147).

[0199] These stacked combinations can be created by any method, including but not limited to cross breeding plants by any conventional or TopCross methodology or genetic transformation. If the traits are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences of interest can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of a polynucleotide of interest. This may be accompanied by any combination of other suppression cassettes or over-expression cassettes to generate the desired combination of traits in the plant.

Use in Breeding Methods

[0200] The transformed plants of the invention may be used in a plant breeding program. The goal of plant breeding is to combine, in a single variety or hybrid, various desirable traits. For field crops, these traits may include, for example, resistance to diseases and insects, tolerance to heat and drought, reduced time to crop maturity, greater yield and better agronomic quality. With mechanical harvesting of many crops, uniformity of plant characteristics such as germination and stand establishment, growth rate, maturity and plant and ear height is desirable. Traditional plant breeding is an important tool in developing new and improved commercial crops. This invention encompasses methods for producing a maize plant by crossing a first parent maize plant with a second parent maize plant wherein one or both of the parent maize plants is a transformed plant displaying a drought tolerance phenotype, a sterility phenotype, a density tolerance phenotype or the like, as described herein.

[0201] Plant breeding techniques known in the art and used in a maize plant breeding program include, but are not limited to, recurrent selection, bulk selection, mass selection, backcrossing, pedigree breeding, open pollination breeding, restriction fragment length polymorphism enhanced selection, genetic marker enhanced selection, doubled haploids and transformation. Often combinations of these techniques are used.

[0202] The development of maize hybrids in a maize plant breeding program requires, in general, the development of homozygous inbred lines, the crossing of these lines and the evaluation of the crosses. There are many analytical methods available to evaluate the result of a cross. The oldest and most traditional method of analysis is the observation of phenotypic traits. Alternatively, the genotype of a plant can be examined.

[0203] A genetic trait which has been engineered into a particular maize plant using transformation techniques can be moved into another line using traditional breeding techniques that are well known in the plant breeding arts. For example, a backcrossing approach is commonly used to move a transgene from a transformed maize plant to an elite inbred line and the resulting progeny would then comprise the transgene (s). Also, if an inbred line was used for the transformation, then the transgenic plants could be crossed to a different inbred in order to produce a transgenic hybrid maize plant. As used herein, "crossing" can refer to a simple X by Y cross or the process of backcrossing, depending on the context.

[0204] The development of a maize hybrid in a maize plant breeding program involves three steps: (1) the selection of plants from various germplasm pools for initial breeding crosses; (2) the selfing of the selected plants from the breeding crosses for several generations to produce a series of inbred lines, which, while different from each other, breed true and are highly homozygous and (3) crossing the selected inbred lines with different inbred lines to produce the hybrids. During the inbreeding process in maize, the vigor of the lines decreases. Vigor is restored when two different inbred lines are crossed to produce the hybrid. An important consequence of the homozygosity and homogeneity of the inbred lines is that the hybrid created by crossing a defined pair of inbreds will always be the same. Once the inbreds that give a superior hybrid have been identified, the hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained.

[0205] Transgenic plants of the present invention may be used to produce, e.g., a single cross hybrid, a three-way hybrid or a double cross hybrid. A single cross hybrid is produced when two inbred lines are crossed to produce the F1 progeny. A double cross hybrid is produced from four inbred lines crossed in pairs (A×B and C×D) and then the two F1 hybrids are crossed again (A×B) times (C×D). A three-way cross hybrid is produced from three inbred lines where two of the inbred lines are crossed (A×B) and then the resulting F1 hybrid is crossed with the third inbred (A×B)×C. Much of the hybrid vigor and uniformity exhibited by F1 hybrids is lost in the next generation (F2). Consequently, seed produced by hybrids is consumed rather than planted.

Kits for Modulating Drought Tolerance or Other Traits

[0206] Certain embodiments of the invention can optionally be provided to a user as a kit. For example, a kit of the invention can contain one or more nucleic acid, polypeptide, antibody, diagnostic nucleic acid or polypeptide, e.g., antibody, probe set, e.g., as a cDNA microarray, one or more vector and/or cell line described herein. Most often, the kit is packaged in a suitable container. The kit typically further comprises one or more additional reagents, e.g., substrates, labels, primers or the like for labeling expression products, tubes and/or other accessories, reagents for collecting samples, buffers, hybridization chambers, cover slips, etc. The kit optionally further comprises an instruction set or user manual detailing preferred methods of using the kit components for discovery or application of gene sets. When used according to the instructions, the kit can be used, e.g., for evaluating expression or polymorphisms in a plant sample, e.g., for evaluating ACC synthase activity, ethylene production, density resistance potential, sterility, etc. Alternatively,

the kit can be used according to instructions for using at least one ACC synthase polynucleotide sequence to modulate drought tolerance in a plant.

[0207] As another example, a kit includes a container containing at least one polynucleotide sequence comprising a nucleic acid sequence, wherein the nucleic acid sequence is, e.g., at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 99.5% or more, identical to SEQ ID NO: 1, 2, 3 or 4 or a subsequence thereof or a complement thereof. The kit optionally also includes instructional materials for the use of the at least one polynucleotide sequence in a plant.

Other Nucleic Acid and Protein Assays

[0208] In the context of the invention, nucleic acids and/or proteins are manipulated according to well known molecular biology methods. Detailed protocols for numerous such procedures are described in, e.g., in Ausubel, et al., Current Protocols in Molecular Biology (supplemented through 2004) John Wiley & Sons, New York ("Ausubel"); Sambrook, et al., Molecular Cloning—A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1989) ("Sambrook") and Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, Calif. ("Berger").

[0209] In addition to the above references, protocols for in vitro amplification techniques, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), useful, e.g., for amplifying polynucleotides of the invention, are found in Mullis, et al., (1987) U.S. Pat. No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis, et al., eds) Academic Press Inc. San Diego, Calif. (1990) ("Innis"); Arnheim and Levinson, (1990) C&EN 36; The Journal Of NIH Research (1991) 3:81; Kwoh, et al., (1989) *Proc Natl Acad Sci USA* 86:1173; Guatelli, et al., (1990) *Proc Natl Acad Sci USA* 87:1874; Lomell, et al., (1989) *J Clin Chem* 35:1826; Landegren, et al., (1988) *Science* 241:1077; Van Brunt, (1990) *Biotechnology* 8:291; Wu and Wallace, (1989) *Gene* 4:560; Barringer, et al., (1990) *Gene* 89:117 and Sooknanan and Malek, (1995) *Biotechnology* 13:563. Additional methods, useful for cloning nucleic acids in the context of the invention, include Wallace, et al., U.S. Pat. No. 5,426,039. Improved methods of amplifying large nucleic acids by PCR are summarized in Cheng, et al., (1994) *Nature* 369:684 and the references therein.

[0210] Certain polynucleotides of the invention can be synthesized utilizing various solid-phase strategies involving mononucleotide- and/or trinucleotide-based phosphoramidite coupling chemistry. For example, nucleic acid sequences can be synthesized by the sequential addition of activated monomers and/or trimers to an elongating polynucleotide chain. See, e.g., Caruthers, et al., (1992) *Meth Enzymol* 211:3. In lieu of synthesizing the desired sequences, essentially any nucleic acid can be custom ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com) (Midland, Tex.), The Great American Gene Company (available on the World Wide Web at genco.com) (Ramona, Calif.), ExpressGen, Inc. (available on the World Wide Web at expressgen.com) (Chicago, Ill.), Operon Technologies, Inc. (available on the World Wide Web at operon.com) (Alameda, Calif.) and many others.

TABLE 1

Sequence Identification.

SEQ ID NO:	Position within SEQ ID NO: 3	DESCRIPTION
1	3272-3776	TR3 ACS6 down-regulation sequence
2	4332-4874	TR4 ACS6 down-regulation sequence
3	1-51, 280	Entire plasmid sequence
4	3272-4874	Comprising TR3, ADH1 intron 1, and TR4
5	1218-4874	Comprising UBI1Zm promoter, UBI1Zm 5'UTR, UBI1Zm Intron 1, TR3, ADH1 intron 1, and TR4
6	1218-7989	Comprising UBI1Zm promoter, UBI1Zm 5'UTR, UBI1Zm Intron 1, TR3, ADH1 intron 1, TR4, ATTB2, FRT12, UBI1Zm promoter, UBI1Zm 5' UTR, UBI1Zm Intron 1, MO-PAT, and PinII terminator
7	1-8350	Complete ACS down-regulation expression cassette
8	n/a	Sorghum bicolor PEP carboxylase promoter
9	n/a	genomic maize ACS3 sequence
10	n/a	UTR and CDS of maize ACS3 sequence
11	n/a	maize ACS3 amino acid sequence
12	n/a	rice ACS6 coding sequence
13	n/a	rice ACS6 amino acid sequence

EXAMPLES

[0211] The following examples are offered to illustrate, but not to limit, the claimed invention. Various modifications by persons skilled in the art are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1

Protein Extraction

[0212] For total protein isolation, maize leaves are collected at the indicated times, quick-frozen in liquid nitrogen and ground to a fine powder. One ml of extraction buffer (20 mM HEPES (pH 7.6), 100 mM KCl, 10% Glycerol) is added to approximately 0.1 g frozen powder and mixed thoroughly. Samples are centrifuged 10 minutes at 10,000 rpm, the supernatant removed to a new tube and the concentration deter-

mined spectrophotometrically according to the methods of Bradford, (1976). See, Bradford, (1976) *Anal. Biochem.* 72:248-254.

Chlorophyll Extraction

[0213] Leaves are frozen in liquid nitrogen and ground to a fine powder. Samples of approximately 0.1 g are removed to a 1.5 ml tube and weighed. Chlorophyll is extracted 5× with 1 ml (or 0.8 ml) of 80% acetone. Individual extractions are combined and the final volume adjusted to 10 ml (or 15 ml) with additional 80% acetone. Chlorophyll content (a+b) is determined spectrophotometrically according to the methods of Wellburn, (1994). See, Wellburn, (1994) *J. Plant Physiol.* 144:307-313.

Measurement of Photosynthesis

[0214] Plants are grown in the field under normal and drought-stress conditions. Under normal conditions, plants are watered with an amount sufficient for optimum growth and yield. For drought-stressed plants, water may be limited for a period starting approximately one week before pollination and continuing through three weeks after pollination. During the period of limited water availability, drought-stressed plants may show visible signs of wilting and leaf rolling. The degree of stress may be calculated as % yield reduction relative to that obtained under well-watered conditions. Transpiration, stomatal conductance and CO_2 assimilation are determined with a portable TPS-1 Photosynthesis System (PP Systems, Amesbury, Mass.). Each leaf on a plant may be measured, e.g. at forty days after pollination. Values typically represent a mean of six determinations.

DNA and RNA Purification

[0215] For total nucleic acid isolation, maize leaves are collected at desired times, quick-frozen in liquid nitrogen and ground to a fine powder. Ten ml of extraction buffer (100 mM Tris (pH 8.0), 50 mM EDTA, 200 mM NaCl, 1% SDS, 10 μl/ml β-mercaptoethanol) is added and mixed thoroughly until thawed. Ten ml of Phenol/Chloroform (1:1, vol:vol) is added and mixed thoroughly. Samples are centrifuged 10 min at 8,000 rpm, the supernatant is removed to a new tube and the nucleic acid is precipitated at −20° C. following addition of ⅒ vol 3M sodium acetate and 1 vol isopropanol. Total nucleic acid is pelleted by centrifugation at 8,000 rpm and resuspended in 1 ml TE. One half of the prep is used for DNA purification and the remaining half is used for RNA purification. Alternatively, DNA or total nucleic acids can be extracted from 1 cm^2 of seedling leaf, quick-frozen in liquid nitrogen and ground to a fine powder. 600 μl of extraction buffer [100 mM Tris (pH 8.0), 50 mM EDTA, 200 mM NaCl, 1% SDS, 10 μl/ml β-mercaptoethanol] is added and the sample mixed. The sample is extracted with 700 μl phenol/chloroform (1:1) and centrifuged for 10 minutes at 12,000 rpm. DNA is precipitated and resuspended in 600 μl H2O.

[0216] For DNA purification, 500 μg Dnase-free Rnase is added to the tube and incubated at 37° C. for 1 hr. Following Rnase digestion, an equal volume of Phenol/Chloroform (1:1, vol:vol) is added and mixed thoroughly. Samples are centrifuged 10 min at 10,000 rpm, the supernatant is removed to a new tube and the DNA precipitated at −20° C. following addition of ⅒ vol 3M sodium acetate and 1 vol isopropanol. DNA is resuspended in sterile water and the concentration is determined spectrophotometrically. To determine DNA integrity, 20 mg of DNA is separated on a 1.8% agarose gel and visualized following staining with ethidium bromide.

[0217] RNA is purified by 2 rounds of $LiCl_2$ precipitation according to methods described by Sambrook, et al., supra.

Real-Time RT-PCR Analysis

[0218] Fifty μg total RNA is treated with RQ1™ DNase (Promega) to ensure that no contaminating DNA is present. Two μg total RNA is used directly for cDNA synthesis using the Omniscript™ reverse transcription kit (Qiagen) with oligo-dT(20) as the primer.

[0219] Analysis of transcript abundance is accomplished using the QuantiTect™ SYBR Green PCR kit (Qiagen). Reactions contain 1× buffer, 0.5 μl of the reverse transcription reaction (equivalent to 50 ng total RNA) and 0.25 μM (final concentration) forward and reverse primers in a total reaction volume of 25 μl.

[0220] Reactions are carried out using an ABI PRISM 7700 sequence detection system under the following conditions: 95° C./115 minutes (1 cycle); 950/30 sec, 62° C./30 sec, 72° C./2 minute (50 cycles); 72° C./5 minutes (1 cycle). Each gene is analyzed a minimum is of four times.

[0221] Primer combinations are initially run and visualized on an agarose gel to confirm the presence of a single product of the correct size. Amplification products are subcloned into the pGEM®-T Easy Vector System (Promega) to use for generation of standard curves to facilitate conversion of expression data to a copy/μg RNA basis.

Ethylene Determination

[0222] Ethylene may be measured from leaves, such as the second fully-expanded leaf of seedlings at the 4-leaf stage or the terminal 15 cm of leaves of plants 20, 30 or 40 days after pollination (DAP). Leaves are harvested at the indicated time or times and allowed to recover between moist paper towels for 2 hours prior to collecting ethylene. Leaves are placed into glass vials and capped with a rubber septum. Following a 3- to 4-hour incubation, 0.9 mL of headspace is sampled from each vial.

[0223] Ethylene may be measured from developing kernels at four time points: 14, 21, 27 and 29 days after pollination (DAP). Kernels are harvested and incubated in well-circulated air for 2 hr to liberate any stress ethylene. Kernels are then placed into 5 mL glass vials and immediately sealed with airtight subaseal stoppers or crimp tops. The vials are then incubated in the dark for 24 h at 28° C. Following this incubation, 0.2 mL of headspace is sampled from each vial.

[0224] Ethylene content is measured using an GC6890 series gas chromatography system with FID detection (Agilent Technologies, Palo Alto, Calif.) with the following parameters: Column J&W Porous Layer Open Tubular (PLOT) with HP-AL/M stationary phase; size 50 m×0.535 mm×15 μm; oven temperature 75° C. isothermal; run time 2 minutes; injector 250° C. splitless, pressure 22 psi. Standards used are from Praxair (Danbury, Conn.) for 10 ppm, 50 ppm and 100 ppm ethylene in an air balance. The limit of detection (LOD) is approximately 0.1 ppm; the limit of quantitation (LOQ) is approximately 0.5 ppm.

Western Blot Analysis

[0225] Leaves are collected at the indicated times and ground in liquid nitrogen to a fine powder. One ml of extraction buffer [20 mM HEPES (pH 7.6), 100 mM KCl, 10%

glycerol, 1 mM PMSF] is added to approximately 0.1 g frozen powder and mixed thoroughly. Cell debris is pelleted by centrifugation at 10,000 rpm for 10 min and the protein concentration determined as described (Bradford, 1976). Antiserum raised against the large subunit of rice Rubisco is obtained from Dr. Tadahiko Mae (Tohoku University, Sendai, Japan). Protein extracts are resolved using standard SDS-PAGE and the protein transferred to 0.22 μm nitrocellulose membrane by electroblotting. Following transfer, the membranes are blocked in 5% milk, 0.01% thimerosal in TPBS (0.1% TWEEN® 20, 13.7 mM NaCl, 0.27 mM KCl, 1 mM Na2HPO4, 0.14 mM KH2PO4) followed by incubation with primary antibodies diluted typically 1:1000 to 1:2000 in TPBS with 1% milk for 1.5 hrs. The blots are then washed twice with TPBS and incubated with goat anti-rabbit horseradish peroxidase-conjugated antibodies (Southern Biotechnology Associates, Inc.) diluted to 1:5000 to 1:10,000 for 1 hr. The blots are washed twice with TPBS and the signal detected typically between 1 to 15 min using chemiluminescence (Amersham Corp).

Example 2

ACC Synthase Down-Regulation by Hairpin RNA Expression

[0226] As noted previously, plant cells and plants can be modified by introduction of an ACC synthase polynucleotide sequence configured for RNA silencing or interference. This example describes hairpin RNA expression cassettes for modifying ethylene production, drought tolerance, NUE, seed or biomass yield, density tolerance or other phenotypes, e.g., in maize. As noted previously, down-regulation of ACC synthase(s), e.g., by hairpin RNA (hpRNA) expression, can result in plants or plant cells having reduced expression (up to and including no detectable expression) of one or more ACC synthases.

[0227] Expression of hpRNA molecules specific for one or more ACC synthase genes (e.g., ACC synthase promoters, other untranslated regions or coding regions) in plants can alter phenotypes such as ethylene production, drought tolerance, density tolerance, seed or biomass yield and/or nitrogen use efficiency of the plants, through RNA interference.

[0228] An hpRNA construct as described herein is generated by linking a ubiquitin promoter to a portion of the coding sequence of an ACS gene, such as the ACS6 gene and its inverted repeat sequence. Each construct is transformed into maize using *Agrobacterium*-mediated transformation techniques or another known transformation method. Nucleic acid molecules and methods for preparing the constructs and transforming maize are as previously described and known in the art; see, e.g., the sections herein entitled "Plant Transformation Methods," "Other Nucleic Acid and Protein Assays" and the following example "Transformation of Maize".

[0229] Expression of hpRNA targeting one or more ACC synthase genes, such as an ACS6 coding sequence, may result in maize plants that display no detrimental effects in vegetative and reproductive growth. Sequence of a plasmid comprising such an hpRNA construct a construct of the invention is provided in SEQ ID NO: 3. FIG. **6** is a schematic of a representative expression cassette; the expression cassette sequence is provided in SEQ ID NO: 7. FIG. **1** provides a listing of features of a plasmid comprising a representative expression cassette.

Example 3

Yield Evaluation—Season 1

[0230] Transformed plants of genetic background #1, comprising the sequence of SEQ ID NO: 4 were evaluated for yield under four environments. Eight reps were grown under flowering stress in Environment 1; 6 reps were grown under grain fill stress in Environment 2; 6 reps were grown under grain fill stress in Environment 3 and 4 reps were grown under rain-fed conditions in Environment 4. Yields were compared with a highly repeated construct null (CN) comprising non-transgenic segregants of plants transformed with the construct. The data are shown in FIGS. **2-5**.

[0231] FIG. **2** shows the yield of transformed plants of the invention under flowering stress in Environment 1. Each bar represents a separate transformation event. Average yield of transgene-negative segregants is shown (139 bu/a) as control (CN). A total of 74% of the events yielded nominally more than the control plants. Plants representing 18 transgenic events outyielded the control at $P<0.10$.

[0232] FIG. **3** shows the yield of transformed plants of the invention under grain-fill stress in Environment 2. Each bar represents a separate transformation event. Average yield of transgene-negative segregants is shown (176 bu/a) as control (CN). Thirteen events out-yielded the CN at $P<0.10$. Of these, eight had also shown significant improvement under flowering stress.

[0233] FIG. **4** shows the yield, as a percent of control, of transformed plants of the invention (indicated by a circle), as well as plants transformed using an alternative ACS6 down-regulation vector (indicated by a square) under grain fill stress in Environment 3. Each data point represents a separate transformation event. NS=not significant. The control plants are bulked transgene-negative segregants. As can be seen, 64% of the events of the invention had significantly superior yield; only 17% of the alternative ACS6 down-regulation events had significantly superior yield, relative to the control.

[0234] FIG. **5** shows the yield, as a percent of control, of transformed plants of the invention (indicated by a circle), as well as plants transformed using an alternative ACS6 down-regulation construct (indicated by a square) under rain-fed conditions in Environment 4. Each data point represents a separate transformation event. NS=not significant. The control plants are bulked transgene-negative segregants. As can be seen, all points exhibiting statistically significant increases in yield represent events as disclosed herein. In addition, all points exhibiting statistically significant decreases in yield are events containing the alternative ACS6 down-regulation construct.

[0235] Without being limited to any particular theory, the construct as herein disclosed provides improvement in yield and other phenotypic traits by modulating ACS expression. For example, inclusion of an intron (e.g., Adh1 intron) within the ACS6 hairpin may modulate ACS6 downregulation within an effective range. Alternatively or additionally, the construct of the invention may impact expression of other genes, for example ACS2 and/or ACS3.

Example 4

Screening of Gaspe Bay Flint Derived Maize Lines Under Nitrogen Limiting Conditions

[0236] Transgenic plants will contain two or three doses of Gaspe Flint-3 with one dose of GS3 (GS3/(Gaspe-3)2× or GS3/(Gaspe-3)3×) and will segregate 1:1 for a dominant transgene. Transgenic GS3×Gaspe T1 seeds and their respective nulls will be planted in 4-inch pots containing TURFACE®, a commercial potting medium and watered four times each day with 1 mM KNO_3 growth medium and

with 2 mM (or higher) KNO_3 growth medium. After emergence, plants will be sampled to determine which are transgenic and which are nulls. At anthesis, plants are harvested and dried in a 70° C. oven for 72 hours and the shoot and ear dry weight determined. Results are analyzed for statistical significance. Expression of a transgene results in plants with improved nitrogen use efficiency in 1 mM KNO_3 when compared to a transgenic null. Increase in biomass, greenness and/or ear size at anthesis indicates increased NUE.

Example 5

NUE Assay

[0237] Seeds of *Arabidopsis thaliana* (control and transgenic line), ecotype Columbia, are surface sterilized (Sánchez, et al., 2002) and then plated on to Murashige and Skoog (MS) medium containing 0.8% (w/v) Bacto™-Agar (Difco). Plates are incubated for 3 days in darkness at 4° C. to break dormancy (stratification) and transferred thereafter to growth chambers (Conviron, Manitoba, Canada) at a temperature of 20° C. under a 16-h light/8-h dark cycle. The average light intensity is 120 μE/m2/s. Seedlings are grown for 12 days and then transferred to soil based pots. Potted plants are grown on a nutrient-free soil LB2 Metro-Mix® 200 (Scott's Sierra Horticultural Products, Marysville, Ohio, USA) in individual 1.5-in pots (Arabidopsis system; Lehle Seeds, Round Rock, Tex., USA) in growth chambers, as described above. Plants are watered with 0.6 or 6.5 mM potassium nitrate in the nutrient solution based on Murashige and Skoog (MS free Nitrogen) medium. The relative humidity is maintained around 70%. Sixteen to eighteen days later, plant shoots are collected for evaluation of biomass and SPAD (chlorophyll) readings.

Example 6

Sucrose Growth Assay

[0238] The Columbia line of *Arabidopsis thaliana* is obtained from the *Arabidopsis* Biological Resource Center (Columbus, Ohio). For early analysis (Columbia and T3 transgenic lines), seed are surface-sterilized with 70% ethanol for 5 minutes followed by 40% Clorox® for 5 minutes and rinsed with sterile deionized water. Surface-sterilized seed are sown onto square Petri plates (25 cm) containing 95 mL of sterile medium consisting of 0.5 Murashige and Skoog (1962) salts (Life Technologies) and 4% (w/v) phytagel (Sigma). The medium contains no supplemental sucrose. Sucrose is added to medium in 0.1%, 0.5% and 1.5% concentration. Plates are arranged vertically in plastic racks and placed in a cold room for 3 days at 4° C. to synchronize germination. Racks with cold stratified seed are then transferred into growth chambers (Conviron, Manitoba, Canada) with day and night temperatures of 22 and 20° C., respectively. The average light intensity at the level of the rosette is maintained at 110 mol/m2/sec1 during a 16-hr light cycle development beginning at removal from the cold room (day 3 after sowing) until the seedlings are harvested on day 14. Images are taken and total fresh weight of root and shoot are measured.

Example 7

Low Nitrogen Seedling Assay Protocol

[0239] Seed of transgenic events are separated into transgene and null seed. Two different random assignments of treatments are made to each block of 54 pots arranged 6 rows of 9 columns using 9 replicates of all treatments. In one case null seed of 5 events of the same construct are mixed and used as control for comparison of the 5 positive events in this block, making up 6 treatment combinations in each block. In the second case, 3 transgenic positive treatments and their corresponding nulls are randomly assigned to the 54 pots of the block, making 6 treatment combinations for each block, containing 9 replicates of all treatment combinations. In the first case transgenic parameters are compared to a bulked construct null and in the second case transgenic parameters are compared to the corresponding event null. In cases where there are 10, or 20 events in a construct, the events are assigned in groups of 5 events, the variances calculated for each block of 54 pots but the block null means pooled across blocks before mean comparisons are made.

[0240] Two seed of each treatment are planted in 4 inch, square pots containing TURFACE®-MVP on 8 inch, staggered centers and watered four times each day with a solution containing the following nutrients:

1 mM $CaCl_2$	2 mM $MgSO_4$	0.5 mM KH_2PO_4	83 ppm Sprint330
3 mM KCl	1 mM KNO_3	1 uM $ZnSO_4$	1 uM $MnCl_2$
3 uM H_3BO_4	1 uM $MnCl_2$	0.1 uM $CuSO_4$	0.1 uM $NaMoO_4$

[0241] After emergence the plants are thinned to one seed per pot. Seedlings are harvested 18 days after planting. At harvest, plants are removed from the pots and the Turface washed from the roots. The roots are separated from the shoot, placed in a paper bag and dried at 70° C. for 70 hr. The dried plant parts (roots and shoots) are weighed and placed in a 50 ml conical tube with approximately 20 5⁄32 inch steel balls and ground by shaking in a paint shaker. Approximately, 30 mg of the ground tissue is hydrolyzed in 2 ml of 20% H_2O_2 and 6M H_2SO_4 for 30 minutes at 170° C. After cooling, water is added to 20 ml, mixed thoroughly, and a 50 μl aliquot removed and added to 950 μl 1 M Na_2CO_3. The ammonia in this solution is used to estimate total reduced plant nitrogen by placing 100 μl of this solution in individual wells of a 96 well plate followed by adding 50 μl of OPA solution. Fluorescence, excitation=360 nM/emission=530 nM, is determined and compared to NH_4Cl standards dissolved in a similar solution and treated with OPA solution.

OPA solution−5 ul Mercaptoethanol+1 ml OPA stock solution

OPA stock−50 mg o-phthadialdehyde(OPA−Sigma #P0657)dissolved in 1.5 ml methanol+4.4 ml 1 M Borate buffer pH9.5 (3.09 g H_3BO_4+1 g NaOH in 50 ml water)+0.55 ml 20% SDS

[0242] The following parameters are measured and means compared to null mean parameters using a Student's t test: total plant biomass; root biomass; shoot biomass; root/shoot ratio; plant N concentration; total plant N.

[0243] Variance is calculated within each block using a nearest neighbor calculation as well as by Analysis of Variance (ANOVA) using a completely random design (CRD) model. An overall treatment effect for each block is calculated using an F statistic by dividing overall block treatment mean square by the overall block error mean square.

Example 8

Transformation of Maize

Biolistics

[0244] Polynucleotides contained within a vector can be transformed into embryogenic maize callus by particle bombardment, generally as described by Tomes, et al., Plant Cell, Tissue and Organ Culture: Fundamental Methods, Eds. Gamborg and Phillips, Chapter 8, pgs. 197-213 (1995) and as briefly outlined below. Transgenic maize plants can be produced by bombardment of embryogenically responsive immature embryos with tungsten particles associated with DNA plasmids. The plasmids typically comprise a selectable marker and a structural gene, or a selectable marker and an ACC synthase downregulation polynucleotide sequence or subsequence, or the like.

Preparation of Particles

[0245] Fifteen mg of tungsten particles (General Electric), 0.5 to 1.8μ, preferably 1 to 1.8μ, and most preferably 1μ, are added to 2 ml of concentrated nitric acid. This suspension is sonicated at 0° C. for 20 minutes (Branson Sonifier Model 450, 40% output, constant duty cycle). Tungsten particles are pelleted by centrifugation at 10000 rpm (Biofuge) for one minute and the supernatant is removed. Two milliliters of sterile distilled water are added to the pellet, and brief sonication is used to resuspend the particles. The suspension is pelleted, one milliliter of absolute ethanol is added to the pellet and brief sonication is used to resuspend the particles. Rinsing, pelleting and resuspending of the particles are performed two more times with sterile distilled water and finally the particles are resuspended in two milliliters of sterile distilled water. The particles are subdivided into 250 μl aliquots and stored frozen.

Preparation of Particle-Plasmid DNA Association

[0246] The stock of tungsten particles are sonicated briefly in a water bath sonicator (Branson Sonifier Model 450, 20% output, constant duty cycle) and 50 μl is transferred to a microfuge tube. The vectors are typically cis: that is, the selectable marker and the gene (or other polynucleotide sequence) of interest are on the same plasmid.

[0247] Plasmid DNA is added to the particles for a final DNA amount of 0.1 to 10 μg in 10 μL total volume and briefly sonicated. Preferably, 10 μg (1 μg/μL in TE buffer) total DNA is used to mix DNA and particles for bombardment. Fifty microliters (50 μL) of sterile aqueous 2.5 M $CaCl_2$ are added and the mixture is briefly sonicated and vortexed. Twenty microliters (20 μL) of sterile aqueous 0.1 M spermidine are added and the mixture is briefly sonicated and vortexed. The mixture is incubated at room temperature for 20 minutes with intermittent brief sonication. The particle suspension is centrifuged and the supernatant is removed. Two hundred fifty microliters (250 μL) of absolute ethanol are added to the pellet, followed by brief sonication. The suspension is pelleted, the supernatant is removed and 60 μl of absolute ethanol are added. The suspension is sonicated briefly before loading the particle-DNA agglomeration onto macrocarriers.

Preparation of Tissue

[0248] Immature embryos of maize variety High Type II are the target for particle bombardment-mediated transformation. This genotype is the F1 of two purebred genetic lines, parents A and B, derived from the cross of two known maize inbreds, A188 and B73. Both parents were selected for high competence of somatic embryogenesis, according to Armstrong, et al., (1991) *Maize Genetics Coop. News* 65:92.

[0249] Ears from F1 plants are selfed or sibbed and embryos are aseptically dissected from developing caryopses when the scutellum first becomes opaque. This stage occurs about 9 to 13 days post-pollination and most generally about 10 days post-pollination, depending on growth conditions. The embryos are about 0.75 to 1.5 millimeters long. Ears are surface sterilized with 20% to 50% Clorox® for 30 minutes, followed by three rinses with sterile distilled water.

[0250] Immature embryos are cultured with the scutellum oriented upward, on embryogenic induction medium comprised of N6 basal salts, Eriksson vitamins, 0.5 mg/l thiamine HCl, 30 gm/l sucrose, 2.88 gm/l L-proline, 1 mg/l 2,4-dichlorophenoxyacetic acid, 2 gm/l Gelrite® and 8.5 mg/l $AgNO_3$. Chu, et al., (1975) *Sci. Sin.* 18:659; Eriksson, (1965) *Physiol. Plant* 18:976. The medium is sterilized by autoclaving at 121° C. for 15 minutes and dispensed into 100×25 mm Petri dishes. $AgNO_3$ is filter-sterilized and added to the medium after autoclaving. The tissues are cultured in complete darkness at 28° C. After about 3 to 7 days, most usually about 4 days, the scutellum of the embryo swells to about double its original size and the protuberances at the coleorhizal surface of the scutellum indicate the inception of embryogenic tissue. Up to 100% of the embryos display this response, but most commonly, the embryogenic response frequency is about 80%.

[0251] When the embryogenic response is observed, the embryos are transferred to a medium comprised of induction medium modified to contain 120 gm/l sucrose. The embryos are oriented with the coleorhizal pole, the embryogenically responsive tissue, upwards from the culture medium. Ten embryos per Petri dish are located in the center of a Petri dish in an area about 2 cm in diameter. The embryos are maintained on this medium for 3 to 16 hours, preferably 4 hours, in complete darkness at 28° C. just prior to bombardment with particles associated with plasmid DNA.

[0252] To effect particle bombardment of embryos, the particle-DNA agglomerates are accelerated using a DuPont PDS-1000 particle acceleration device. The particle-DNA agglomeration is briefly sonicated and 10 μl are deposited on macrocarriers and the ethanol is allowed to evaporate. The macrocarrier is accelerated onto a stainless-steel stopping screen by the rupture of a polymer diaphragm (rupture disk). Rupture is effected by pressurized helium. The velocity of particle-DNA acceleration is determined based on the rupture disk breaking pressure. Rupture disk pressures of 200 to 1800 psi are used, with 650 to 1100 psi being preferred and about 900 psi being most highly preferred. Multiple disks are used to effect a range of rupture pressures.

[0253] The shelf containing the plate with embryos is placed 5.1 cm below the bottom of the macrocarrier platform (shelf #3). To effect particle bombardment of cultured immature embryos, a rupture disk and a macrocarrier with dried particle-DNA agglomerates are installed in the device. The He pressure delivered to the device is adjusted to 200 psi above the rupture disk breaking pressure. A Petri dish with the target embryos is placed into the vacuum chamber and located in the projected path of accelerated particles. A vacuum is created in the chamber, preferably about 28 in Hg. After operation of the device, the vacuum is released and the Petri dish is removed.

[0254] Bombarded embryos remain on the osmotically-adjusted medium during bombardment, and 1 to 4 days subsequently. The embryos are transferred to selection medium comprised of N6 basal salts, Eriksson vitamins, 0.5 mg/l thiamine HCl, 30 gm/l sucrose, 1 mg/l 2,4-dichlorophenoxyacetic acid, 2 gm/l Gelrite®, 0.85 mg/l Ag NO_3 and 3 mg/l bialaphos (Herbiace, Meiji). Bialaphos is added filter-sterilized. The embryos are subcultured to fresh selection medium at 10 to 14 day intervals. After about 7 weeks, embryogenic tissue, putatively transformed for both selectable and unselected marker genes, proliferates from a fraction of the bombarded embryos. Putative transgenic tissue is rescued and that tissue derived from individual embryos is considered to be an event and is propagated independently on selection medium. Two cycles of clonal propagation are achieved by visual selection for the smallest contiguous fragments of organized embryogenic tissue.

[0255] A sample of tissue from each event is processed to recover DNA. The DNA is restricted with a restriction endonuclease and probed with primer sequences designed to amplify DNA sequences overlapping the ACC synthase and non-ACC synthase portion of the plasmid. Embryogenic tissue with amplifiable sequence is advanced to plant regeneration.

[0256] For regeneration of transgenic plants, embryogenic tissue is subcultured to a medium comprising MS salts and vitamins (Murashige and Skoog, (1962) *Physiol. Plant* 15:473), 100 mg/l myo-inositol, 60 gm/l sucrose, 3 gm/l Gelrite®, 0.5 mg/l zeatin, 1 mg/l indole-3-acetic acid, 26.4 ng/l cis-trans-abscissic acid and 3 mg/l bialaphos in 100×25 mm Petri dishes and is incubated in darkness at 28° C. until the development of well-formed, matured somatic embryos is seen. This requires about 14 days. Well-formed somatic embryos are opaque and cream-colored and are comprised of an identifiable scutellum and coleoptile. The embryos are individually subcultured to a germination medium comprising MS salts and vitamins, 100 mg/l myo-inositol, 40 gm/l sucrose and 1.5 gm/l Gelrite® in 100×25 mm Petri dishes and incubated under a 16 hour light:8 hour dark photoperiod and 40 meinsteinsm^{-2}sec^{-1} from cool-white fluorescent tubes. After about 7 days, the somatic embryos germinate and produce a well-defined shoot and root. The individual plants are subcultured to germination medium in 125×25 mm glass tubes to allow further plant development. The plants are maintained under a 16 hour light: 8 hour dark photoperiod and 40 meinsteinsm^{-2}sec^{-1} from cool-white fluorescent tubes. After about 7 days, the plants are well-established and are transplanted to horticultural soil, hardened off and potted into commercial greenhouse soil mixture and grown to sexual maturity in a greenhouse. An elite inbred line is used as a male to pollinate regenerated transgenic plants.

Agrobacterium-Mediated

[0257] For *Agrobacterium*-mediated transformation, the method of Zhao, et al., may be employed as in PCT Patent Publication Number WO 1998/32326, the contents of which are hereby incorporated by reference. Briefly, immature embryos are isolated from maize and the embryos contacted with a suspension of *Agrobacterium* (step 1: the infection step). In this step the immature embryos are preferably immersed in an *Agrobacterium* suspension for the initiation of inoculation. The embryos are co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step). Preferably the immature embryos are cultured on solid medium following the infection step. Following this co-cultivation period an optional "resting" step is contemplated. In this resting step, the embryos are incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformants (step 3: resting step). Preferably the immature embryos are cultured on solid medium with antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for the infected cells. Next, inoculated embryos re cultured on medium containing a selective agent and growing transformed callus is recovered (step 4: the selection step). Preferably, the immature embryos are cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus is then regenerated into plants (step 5: the regeneration step) and preferably calli grown on selective medium are cultured on solid medium to regenerate the plants.

Example 9

Expression of Transgenes in Monocots

[0258] A plasmid vector is constructed comprising a preferred promoter operably linked to an isolated polynucleotide comprising an ACC synthase polynucleotide sequence or subsequence. This construct can then be introduced into maize cells by the following procedure.

[0259] Immature maize embryos are dissected from developing caryopses derived from crosses of maize lines. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu, et al., (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27° C. Friable embryogenic callus, consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures, proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

[0260] The plasmid p35S/Ac (Hoechst Ag, Frankfurt, Germany) or equivalent may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the Pat gene (see, EP Patent Publication Number 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The pat gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell, et al., (1985) *Nature* 313:810-812) and comprises the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

[0261] The particle bombardment method (Klein, et al., (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed

again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He biolistic particle delivery system (Bio-Rad Instruments, Hercules, Calif.), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

[0262] For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covers a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

[0263] Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

[0264] Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm, et al., (1990) Bio/Technology 8:833-839).

Example 10

Expression of Transgenes in Dicots

[0265] Soybean embryos are bombarded with a plasmid comprising a preferred promoter operably linked to a heterologous nucleotide sequence comprising an ACC synthase polynucleotide sequence or subsequence (e.g., SEQ ID NOS: 1 and 2), as follows. To induce somatic embryos, cotyledons of 3 to 5 mm in length are dissected from surface-sterilized, immature seeds of the soybean cultivar A2872, then cultured in the light or dark at 26° C. on an appropriate agar medium for six to ten weeks. Somatic embryos producing secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos that multiply as early, globular-staged embryos, the suspensions are maintained as described below.

[0266] Soybean embryogenic suspension cultures can be maintained in 35 ml liquid media on a rotary shaker, 150 rpm, at 26° C. with fluorescent lights on a 16:8 hour day/night schedule. Cultures are sub-cultured every two weeks by inoculating approximately 35 mg of tissue into 35 ml of liquid medium.

[0267] Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein, et al., (1987) *Nature* (*London*) 327:70-73, U.S. Pat. No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

[0268] A selectable marker gene that can be used to facilitate soybean transformation is a transgene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell, et al., (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz, et al., (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens. The expression cassette of interest, comprising the preferred promoter and a heterologous ACC synthase polynucleotide, can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.*

[0269] To 50 μl of a 60 mg/ml 1 μm gold particle suspension is added (in order): 5 μl DNA (1 μg/μl), 20 μl spermidine (0.1 M) and 50 μl $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μl 70% ethanol and resuspended in 40 μl of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five microliters of the DNA-coated gold particles are then loaded on each macro carrier disk.

[0270] Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60×5 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi, and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

[0271] Five to seven days post bombardment, the liquid media may be exchanged with fresh media and eleven to twelve days post-bombardment with fresh media containing 50 mg/ml hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post-bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

Example 11

Field Trials Under Nitrogen Stress and Normal Nitrogen Conditions

[0272] Corn hybrids containing an ACS down-regulation construct transgene are planted in the field under nitrogen-stress and normal-nitrogen conditions. Under normal nitrogen, a total of 250 lbs nitrogen is applied in the form of urea ammonium nitrate (UAN). Nitrogen stress is achieved through depletion of soil nitrogen reserves by planting corn with no added nitrogen for two years. Soil nitrate reserves are monitored to assess the level of depletion. To achieve the target level of stress, UAN is applied by fertigation or sidedress between V2 and VT growth stages, for a total of 50-150 lbs nitrogen.

[0273] Events from the construct are nested together with the null to minimize the spatial effects of field variation. Multiple reps are planted. The seed yield of events containing

the transgene is compared to the yield of a transgenic null. Statistical analysis is conducted to assess whether there is a significant improvement in yield compared with the transgenic null, taking into account row and column spatial effects.

[0274] Differences in yield, yield components or other agronomic traits between transgenic and non-transgenic plants in reduced-nitrogen fertility plots may indicate improvement in nitrogen utilization efficiency contributed by expression of a transgenic event. Similar comparisons are made in plots supplemented with recommended nitrogen fertility rates. Effective transgenic events may achieve similar yields in the nitrogen-limited and normal nitrogen environments or may perform better than the non-transgenic counterpart in low-nitrogen environments.

Example 12

ACS6 Down-Regulation Construct Improves Yield in Reduced-Nitrogen Conditions

[0275] Plants comprising a downregulation construct comprising SEQ ID NO: 4 were planted in the field under nitrogen-stress and normal-nitrogen conditions. Nitrogen stress was achieved through targeted depletion of soil nitrogen reserves by previous corn production and/or limited application of nitrogen fertilitzer. In addition to cropping history, soil type and other environmental factors were taken into consideration in creating appropriate nitrogen-stress conditions.

[0276] The grain yield of plants containing the transgene was compared to the yield of a wild-type or transgenic null. The test used a randomized complete block design with six replications. Statistical analysis was conducted using ASRemI to assess differences in yield, taking into account row and column spatial effects and autoregressive (AR1) adjustments.

[0277] Table 2 provides yield data in bushels/acre for plants representing 19 transformation events under nitrogen-stress conditions in two geographic locations. Yields marked with an asterisk are significantly greater than the control at P<0.1.

TABLE 2

Event	Location 1	Location 2
2.12	121	202*
2.29	124*	199
2.32	123	211*
113.2.7	124*	206*
4.3	124*	204*
4.8	125*	203*
1.23	127*	208*
1.44	126*	207*
2.15	124*	205*
2.2	124	201
2.24	124*	198
2.38	125*	204*
2.49	123	202*
1.14	125*	210*
2.18	126*	206*
2.22	124*	208*
2.8	125*	205*
2.1	125*	206*
66.2.7	124*	202*
Control	120	197

[0278] Additional measurements were taken at Location 2, as follows. Average yield of the transgenic plants under normal-nitrogen conditions was 232 bushels per acre; under nitrogen-stress conditions, the average yield was 203 bushels per acre. Under nitrogen stress, growing-degree-units to pollen shed was 1273 compared to 1330 under normal-nitrogen conditions. In addition, plants grown in the nitrogen-stress environment showed a reduction in anthesis-silking interval (ASI) of 18. Barren count in the low-nitrogen environment was 1 on a 1 to 10 scale, where 10 is least favorable.

Example 13

Yield Evaluation—Season 2

[0279] Maize hybrids were generated by crossing tester lines with plants comprising an ACS downregulation construct substantially as described in FIG. **6**. These plants represented nine separate transformation events in Background 1. Hybrids were evaluated for yield in multiple locations in Season 2. Grain yield was compared to that of untransformed hybrids (WT, wild-type) and bulked nontransgenic segregants (BN, bulk null) from all constructs in the block sharing the same background. Results are shown in FIG. **9**.

Example 14

Yield Evaluation—Season 3

[0280] Four maize hybrids were generated by crossing tester lines with plants comprising an ACS downregulation construct substantially as described in FIG. **6**. Twelve separate transformation events were individually represented in each hybrid combination. The hybrids were grown in a split-plot experimental design with four replications in each of four testing sites. Bulked nontransgenic segregants (BN, bulk null) from all constructs in a block, and/or untransformed hybrids (WT, wild-type), served as controls at each site. Grain yield (bushels/acre) and plant height data were collected and analyzed. Significance was determined at P<0.1 using BLUP (Best Linear Unbiased Predictor) analysis (Henderson, (1975) *Biometrics* 31(2):423-447; Robinson, (1991) *Statistical Science* 6(1):15-32). As shown in FIG. **10**, ten of twelve transgenic events yielded more than both the bulk null and wild-type controls.

Example 15

[0281] ACC synthase (ACS) catalyzes the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) from S-adenosyl-L-methionine (SAM), the first committed step of ethylene biosynthesis. This step is rate-limiting for ethylene formation; expression of ACS is tightly regulated at both the transcriptional and post-transcriptional levels (review, Kende, (1993) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 44:283-307).

[0282] In *Arabidopsis*, 12 genes were named as AtACS. Later AtACS3 was identified as a pseudogene and AtACS10 and AtACS12 were found to encode not ACS but aminotransferases (Yamagami, et al., (2003) *Journal of Biol. Chem.* 278(49):49102-49112. In maize, three ACC synthases (ZmACS6, ZmACS2 and ZmACS7) have been previously studied (Gallie and Young, (2004) *Mol. Gen. Gen.* 271:267-281; Wang, et al., (2002) *Plant Cell* 14:S131-151). A previously unreported maize ACC synthase, designated ZmACS3, is shown in SEQ ID NOS: 9-11. Modulation of expression of ZmACS3, particularly downregulation of ZmACS3, alone or in combination with modulation of other genes, may reduce ethylene production, resulting in increased growth rate and improved stress tolerance in plants. For example, suppression of expression of both ZmACS6 and ZmACS3 in maize may

result in higher growth rate and improved yield under optimal and/or stress (e.g. drought) conditions.

[0283] Methods and compositions to modulate plant development may use DNA, RNA or protein of or derived from the ZmACS3 gene. Certain embodiments provide an isolated polypeptide comprising an amino acid sequence selected from the group consisting of: (a) the polypeptide comprising the amino acid sequence of SEQ ID NO:11; (b) a polypeptide having at least 80% sequence identity to the full length of SEQ ID NO: 11, wherein the polypeptide has ACC synthase activity; (c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising the complement of SEQ ID NO: 10, wherein the stringent conditions comprise 50% formamide, 1 M NaCl, 1% SDS at 37° C. and a wash in 0.1×SSC at 60° C. to 65° C. and (d) the polypeptide having at least 70 consecutive amino acids of SEQ ID NO:11, wherein the polypeptide retains ACC synthase activity.

[0284] The ACS3 polypeptide shares moderate (59%) identity with the ACS6 protein; see, FIG. **14** for an alignment. Identity between the cDNA sequences of ACS6 and ACS3 is approximately 66%; see, FIG. **15** for an alignment.

[0285] By "ACS3 activity" or "ACC Synthase 3 activity" is intended the ACS3 polypeptide has exemplary activity, such as in catalyzing a step in ethylene synthesis. Methods to assay for such activity are known in the art and are described more fully herein. Depending on context, "ACS3 activity" may refer to the activity of a native ACS3 polynucleotide or polypeptide. Such native activity may be modulated by expression of a heterologous ZmACS3 sequence as provided herein, for example when provided in a construct which downregulates the native ZmACS3.

[0286] The level of the ACS3 polypeptide may be measured directly, for example, by assaying for the level of the ACS3 polypeptide in the plant, or indirectly, for example, by measuring the ACS3 activity of the ACS3 polypeptide in the plant. Methods for determining the presence of ACS3 activity are described elsewhere herein or known in the art.

[0287] It is also recognized that the level and/or activity of the polypeptide may be modulated by employing a polynucleotide that is not capable of directing, in a transformed plant, the expression of a protein or an RNA. For example, the polynucleotides of the invention may be used to design polynucleotide constructs that can be employed in methods for altering or mutating a genomic nucleotide sequence, or its expression, in an organism. Such polynucleotide constructs include, but are not limited to, RNA:DNA vectors, RNA:DNA mutational vectors, RNA:DNA repair vectors, mixed-duplex oligonucleotides, self-complementary RNA:DNA oligonucleotides and recombinogenic oligonucleobases. Such nucleotide constructs and methods of use are known in the art. See, U.S. Pat. Nos. 5,565,350; 5,731,181; 5,756,325; 5,760,012; 5,795,972 and 5,871,984, all of which are herein incorporated by reference. See also, PCT Application Publication Numbers WO 98/49350, WO 99/07865, WO 99/25821 and Beetham, et al., (1999) *Proc. Natl. Acad. Sci. USA* 96:8774-8778, herein incorporated by reference.

[0288] The Zm-ACS3 nucleotide sequence set forth in SEQ ID NO: 10 can be used to generate variant nucleotide sequences having the nucleotide sequence of the open reading frame with about 70%, 75%, 80%, 85%, 90% or 95% nucleotide sequence identity when compared to the starting unaltered ORF nucleotide sequence of SEQ ID NO: 10. These functional variants are generated using a standard codon table. While the nucleotide sequence of the variant is altered, the amino acid sequence encoded by the open reading frame does not change.

[0289] Certain embodiments include plants having a transgene comprising a polynucleotide operably linked to a heterologous promoter that drives expression in the plant, wherein expression of the transgene results in modulation of expression of an ACS3 polynucleotide and/or polypeptide. Modulation of expression of other genes, including other ACS genes, may occur as a result of expression of the same transgene or a different transgene. Expression of the transgene may be constitutive or may be directed preferentially to a particular plant cell type or plant tissue type or may be inducible or otherwise controlled. Methods are provided to modulate plant growth and development, particularly plant response to stress, particularly abiotic stress, relative to a control plant, control plant cell or control plant part. The modulated growth or development may be reflected in, for example, higher growth rate, higher yield, altered morphology or appearance and/or an altered response to stress including an improved tolerance to stress. In certain embodiments, the stress is cold, salt or drought. In certain embodiments, yield is increased or maintained during periods of abiotic stress. Yield may be measured, for example, in terms of seed yield, plant biomass yield or recovery of other plant product or products. Seed set may be measured by, for example, seed number, total seed mass, average seed mass or some combination of these or other measures.

Example 16

Down-Regulation of Multiple ACS Genes

[0290] As shown in FIGS. **10-13**, plants comprising a hpRNA comprising SEQ ID NO: 4 have shown consistent yield improvement under drought conditions across multiple environment and years, as well as consistent increase in height. This hpRNA comprises regions of identity to both ZmACS6 and the recently identified ZmACS3 gene. (See, FIG. **16**; underlining indicates identical bases.) In one such region, 44 contiguous nucleotides of the ZmACS3 gene are identical to the hairpin sequence, indicating that the hpRNA of SEQ ID NO: 4 may down-regulate expression of ACS3 as well as ACS6. Further, ZmACS2 and ZmACS7 share contiguous 24- and 25-bp identity regions, respectively, with the hairpin sequence, which may result in downregulation of expression.

Example 17

Yield Evaluation of Transgenic Events in Two Backgrounds and Three Watering Regimes—Season 4

[0291] Hybrid maize plants, created by crossing four tester lines to plants of genetic background 2 or background 3 which comprised the recombinant polynucleotide of SEQ ID NO: 4 were evaluated for yield under three watering regimes in a randomized, nested experiment. Treatments were flowering stress (6 replications), grain-fill stress (4 replications) and well-watered conditions (4 replications). Flowering stress provided a 61% yield reduction, while grain-fill stress provided a 47% yield reduction, both relative to the well-watered yields. Controls were bulked null segregants as described above (BN), and nontransgenic hybrids of comparable base genetics (WT). Significance was determined at $P<0.1$ using BLUP (Best Linear Unbiased Predictor) analysis. Results are

shown in FIG. **13**. Events yielding significantly more than the control are shaded. Non-shaded data are not significantly different from the control values. For background #2, all thirteen events yielded significantly more than the control. For background #3, eleven of thirteen events yielded significantly more than the control; the other two had yield results not statistically different from the control.

Example 18

Reduction of ACC

[0292] Downregulation of ACC synthase may be reflected in reduced levels of ACC. ACC may be assayed, for example, as described in Methods in ant Biochemistry and Molecular Biology (1997) CRC Press, Ed. W. Dashek, at Chapter 12, pp. 158-159. Root tissue of maize plants at stage VT (Iowa State University Cooperative Extension Special Report No. 48 (1982, 1993)) were evaluated for ACC level. Plants of Background 1 comprising an ACS downregulation construct showed reduced ACC levels relative to the control; see FIG. **17**.

Example 19

Reduced Expression of ACS6

[0293] Using quantitative rtPCR methods generally as described elsewhere herein, maize plants transgenic for an ACS6 downregulation construct comprising SEQ ID NO: 4 were analyzed for ACS6 expression. Root tissue of flooded seedlings was sampled and results were as shown in FIG. **18**. Each data point represents twelve plants in three replications at growth stage V3. Events are as indicated (TG) and data for wild type (WT) plants are provided as control.

[0294] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above can be used in various combinations. All publications, patents, patent applications and/or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application and/or other document were individually indicated to be incorporated by reference for all purposes.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 13

<210> SEQ ID NO 1
<211> LENGTH: 505
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 1

tagcagacgc ggaaccagcc gggctcccgg cagtggcagg aggagcccgg ggagatgttg 60

agccccacct cgaagaccac cctcttccac agctccatct cgccctcgaa cgaccggctc 120

cgcatcaggc gccgcatgtt gacccagcag aagagccccg cgttgctctc caggcactcg 180

atgcccacgg ccgccaggcc ctccgccagc tgctcgcgcc gctccctgat ccgccgcgtg 240

ttctccgcga tgtacctccg cgtgaagtcc ctgtcgccca ggagcgacgc caggaggtgc 300

tgcgtctggg acgacaccag gccgaagctc gacatcttgg tggccgcgga gaccacgccg 360

gcgttggacg agtagatggc gcccacgcgg aaccccggga ggcccaggtc cttggacagg 420

ctgtacacca cgtgcacgcg gtccgacagc ggcccaacgc cgacgacgcc gtcgtccgtg 480

gcggcgcgcg cggccaccac ctgca 505

<210> SEQ ID NO 2
<211> LENGTH: 543
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 2

cccggtacgc gccgccacgg acgacggcgt cgtcggcgtt gggccgctgt cggaccgcgt 60

gcacgtggtg tacagcctgt ccaaggacct gggcctcccg gggttccgcg tgggcgccat 120

ctactcgtcc aacgccggcg tggtctccgc ggccaccaag atgtcgagct tcggcctggt 180

gtcgtcccag acgcagcacc tcctggcgtc gctcctgggc gacagggact tcacgcggag 240

-continued

gtacatcgcg gagaacacgc ggcggatcag ggagcggcgc gagcagctgg cggagggcct 300

ggcggccgtg ggcatcgagt gcctggagag caacgcgggg ctcttctgct gggtcaacat 360

gcggcgcctg atgcggagcc ggtcgttcga gggcgagatg gagctgtgga agagggtggt 420

cttcgaggtg gggctcaaca tctccccggg ctcctcctgc cactgccggg agcccggctg 480

gttccgcgtc tgctaaaggg cgaattccag cacactggcg gccgttacta gtggatccga 540

gct 543

<210> SEQ ID NO 3
<211> LENGTH: 51280
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid as shown in Figure 1

<400> SEQUENCE: 3

gtttacccgc caatatatcc tgtcaaacac tgatagttta aactgaaggc gggaaacgac 60

aatctgatca tgagcggaga attaagggag tcacgttatg acccccgccg atgacgcggg 120

acaagccgtt ttacgtttgg aactgacaga accgcaacgt tgaaggagcc actcagcaag 180

ctggtacgat tgtaatacga ctcactatag ggcgaattga gcgctgttta aacgctcttc 240

aactggaaga gcggttacta ccggctggat ggcggggcct tgatcgtgca ccgccggcgt 300

ccggataagt gactagggtc acgtgaccct agtcacttat cgagctagtt accctatgag 360

gtgacatgaa gcgctcacgg ttactatgac ggttagcttc acgactgttg gtggcagtag 420

cgtacgactt agctatagtt ccggtagatc tgaagttcct attccgaagt tcctattctt 480

caaaaggtat aggaacttcc tcgaattgtt gtggtggggt atagaggttt gatataggtg 540

gaactgctgt agagcgtgga gatatagggg gaaagagaac gctgatgtga caagtgagtg 600

agatataggg ggagaaattt agggggaacg ccgaacacag tctaaagaag cttgggaccc 660

aaagcactct gttcgggggt ttttttttt gtctttcaac tttttgctgt aatgttattc 720

aaaataagaa aagcacttgg catggctaag aaatagagtt caacaactga acagtacagt 780

gtattatcaa tggcataaaa aacaaccctt acagcattgc cgtattttat tgatcaaaca 840

ttcaactcaa cactgacgag tggtcttcca ccgatcaacg gactaatgct gctttgtcag 900

atcaccggtt aagtgactag ggtcacgtga ccctagtcac ttaggttacc agagctggtc 960

acctttgtcc accaagatgg aactgcggcc gctcattaat taagtcaggc gcgcctctag 1020

ttgaagacac gttcatgtct tcatcgtaag aagacactca gtagtcttcg gccagaatgg 1080

ccatctggat tcagcaggcc tagaaggcca tttaaatcct gaggatctgg tcttcctaag 1140

gacccgggat atcacaagtt tgtacaaaaa agcaggctcc ggccagagtt acccggaccg 1200

aagcttgcat gcctgcagtg cagcgtgacc cggtcgtgcc cctctctaga gataatgagc 1260

attgcatgtc taagttataa aaaattacca tatttttttt ttgtcacact tgtttgaagt 1320

gcagtttatc tatctttata catatattta aactttactc tacgaataat ataatctata 1380

gtactacaat aatatcagtg ttttagagaa tcatataaat gaacagttag acatggtcta 1440

aaggacaatt gagtattttg acaacaggac tctacagttt tatcttttta gtgtgcatgt 1500

gttctccttt ttttttgcaa atagcttcac ctatataata cttcatccat tttattagta 1560

catccattta gggtttaggg ttaatggttt ttatagacta atttttttag tacatctatt 1620

-continued

ttattctatt ttagcctcta aattaagaaa actaaaactc tattttagtt tttttattta 1680

ataatttaga tataaaatag aataaaataa agtgactaaa aattaaacaa atacccttta 1740

agaaattaaa aaaactaagg aaacattttt cttgtttcga gtagataatg ccagcctgtt 1800

aaacgccgtc gacgagtcta acggacacca accagcgaac cagcagcgtc gcgtcgggcc 1860

aagcgaagca gacggcacgg catctctgtc gctgcctctg gacccctctc gagagttccg 1920

ctccaccgtt ggacttgctc cgctgtcggc atccagaaat tgcgtggcgg agcggcagac 1980

gtgagccggc acggcaggcg gcctcctcct cctctcacgg caccggcagc tacgggggat 2040

tcctttccca ccgctccttc gctttccctt cctcgcccgc cgtaataaat agacacccca 2100

tccacaccct ctttccccaa cctcgtgttg ttcggagcgc acacacacac aaccagatct 2160

cccccaaatc cacccgtcgg cacctccgct tcaaggtacg ccgctcgtcc tcccccccca 2220

ccctctctac cttctctaga tcggcgttcc ggtccatgca tggttagggc ccggtagttc 2280

tacttctgtt catgtttgtg ttagatccgt gtttgtgtta gatccgtgct gctagcgttc 2340

gtacacggat gcgacctgta cgtcagacac gttctgattg ctaacttgcc agtgtttctc 2400

tttggggaat cctgggatgg ctctagccgt tccgcagacg ggatcgattt catgattttt 2460

tttgtttcgt tgcatagggt ttggtttgcc cttttccttt atttcaatat atgccgtgca 2520

cttgtttgtc gggtcatctt ttcatgcttt tttttgtctt ggttgtgatg atgtggtctg 2580

gttgggcggt cgttctagat cggagtagaa ttctgtttca aactacctgg tggatttatt 2640

aattttggat ctgtatgtgt gtgccataca tattcatagt tacgaattga agatgatgga 2700

tggaaatatc gatctaggat aggtatacat gttgatgcgg gttttactga tgcatataca 2760

gagatgcttt ttgttcgctt ggttgtgatg atgtggtgtg gttgggcggt cgttcattcg 2820

ttctagatcg gagtagaata ctgtttcaaa ctacctggtg tatttattaa ttttggaact 2880

gtatgtgtgt gtcatacatc ttcatagtta cgagtttaag atggatggaa atatcgatct 2940

aggataggta tacatgttga tgtgggtttt actgatgcat atacatgatg gcatatgcag 3000

catctattca tatgctctaa ccttgagtac ctatctatta taataaacaa gtatgtttta 3060

taattatttt gatcttgata tacttggatg atggcatatg cagcagctat atgtggattt 3120

ttttagccct gccttcatac gctatttatt tgcttggtac tgtttctttt gtcgatgctc 3180

accctgttgt ttggtgttac ttctgcaggt cgactttaac ttagcctagg atccactagt 3240

aacggccgcc agtgtgctgg aattcgccct ttagcagacg cggaaccagc cgggctcccg 3300

gcagtggcag gaggagcccg gggagatgtt gagccccacc tcgaagacca ccctcttcca 3360

cagctccatc tcgccctcga acgaccggct ccgcatcagg cgccgcatgt tgacccagca 3420

gaagagcccc gcgttgctct ccaggcactc gatgcccacg gccgccaggc cctccgccag 3480

ctgctcgcgc cgctccctga tccgccgcgt gttctccgcg atgtacctcc gcgtgaagtc 3540

cctgtcgccc aggagcgacg ccaggaggtg ctgcgtctgg gacgacacca ggccgaagct 3600

cgacatcttg gtggccgcgg agaccacgcc ggcgttggac gagtagatgg cgcccacgcg 3660

gaaccccggg aggcccaggt ccttggacag gctgtacacc acgtgcacgc ggtccgacag 3720

cggcccaacg ccgacgacgc cgtcgtccgt ggcggcgcgc gcggccacca cctgcagtcg 3780

acgtgcaaag gtccgccttg tttctcctct gtctcttgat ctgactaatc ttggtttatg 3840

attcgttgag taattttggg gaaagcttcg tccacagttt tttttcgatg aacagtgccg 3900

-continued

cagtggcgct gatcttgtat gctatcctgc aatcgtggtg aacttatttc ttttatatcc 3960

tttactccca tgaaaaggct agtaatcttt ctcgatgtaa catcgtccag cactgctatt 4020

accgtgtggt ccatccgaca gtctggctga acacatcata cgatctatgg agcaaaaatc 4080

tatcttccct gttctttaat gaaggacgtc attttcatta gtatgatcta ggaatgttgc 4140

aacttgcaag gaggcgtttc tttctttgaa tttaactaac tcgttgagtg gccctgtttc 4200

tcggacgtaa ggcctttgct gctccacaca tgtccattcg aattttaccg tgtttagcaa 4260

gggcgaaaag tttgcatctt gatgatttag cttgactatg cgattgcttt cctggacccg 4320

tgcagctgga tcccggtacg cgccgccacg gacgacggcg tcgtcggcgt tgggccgctg 4380

tcggaccgcg tgcacgtggt gtacagcctg tccaaggacc tgggcctccc ggggttccgc 4440

gtgggcgcca tctactcgtc caacgccggc gtggtctccg cggccaccaa gatgtcgagc 4500

ttcggcctgg tgtcgtccca gacgcagcac ctcctggcgt cgctcctggg cgacagggac 4560

ttcacgcgga ggtacatcgc ggagaacacg cggcggatca gggagcggcg cgagcagctg 4620

gcggagggcc tggcggccgt gggcatcgag tgcctggaga gcaacgcggg gctcttctgc 4680

tgggtcaaca tgcggcgcct gatgcggagc cggtcgttcg agggcgagat ggagctgtgg 4740

aagagggtgg tcttcgaggt ggggctcaac atctccccgg gctcctcctg ccactgccgg 4800

gagcccggct ggttccgcgt ctgctaaagg gcgaattcca gcacactggc ggccgttact 4860

agtggatccg agctcgaatt ccggtccggg tcacccggtc cgggcctaga aggccgatct 4920

cccgggcacc cagctttctt gtacaaagtg gtgatatcgg accgattaaa ctttaattcg 4980

gtccgatgca tgtatacgaa gttcctattc cgaagttcct attctacata gagtatagga 5040

acttcacctg gtggcgccgc tagtggatcc cccgggctgc agtgcagcgt gacccggtcg 5100

tgcccctctc tagagataat gagcattgca tgtctaagtt ataaaaaatt accacatatt 5160

ttttttgtca cacttgtttg aagtgcagtt tatctatctt tatacatata tttaaacttt 5220

actctacgaa taatataatc tatagtacta caataatatc agtgttttag agaatcatat 5280

aaatgaacag ttagacatgg tctaaaggac aattgagtat tttgacaaca ggactctaca 5340

gttttatctt tttagtgtgc atgtgttctc ctttttttttt gcaaatagct tcacctatat 5400

aatacttcat ccattttatt agtacatcca tttagggttt agggttaatg gtttttatag 5460

actaattttt ttagtacatc tattttattc tattttagcc tctaaattaa gaaaactaaa 5520

actctatttt agtttttttа tttaataatt tagatataaa atagaataaa ataaagtgac 5580

taaaaattaa acaaatаccc tttaagaaat taaaaaaact aaggaaacat ttttcttgtt 5640

tcgagtagat aatgccagcc tgttaaacgc cgtcgacgag tctaacggac accaaccagc 5700

gaaccagcag cgtcgcgtcg ggccaagcga agcagacggc acggcatctc tgtcgctgcc 5760

tctggacccc tctcgagagt tccgctccac cgttggactt gctccgctgt cggcatccag 5820

aaattgcgtg gcggagcggc agacgtgagc cggcacggca ggcggcctcc tcctcctctc 5880

acggcaccgg cagctacggg ggattccttt cccaccgctc cttcgctttc ccttcctcgc 5940

ccgccgtaat aaatagacac cccctccaca ccctctttcc ccaacctcgt gttgttcgga 6000

gcgcacacac acacaaccag atctcccccа aatccacccg tcggcacctc cgcttcaagg 6060

tacgccgctc gtcctccccc ccccccctct ctaccttctc tagatcggcg ttccggtcca 6120

tgcatggtta gggcccggta gttctacttc tgttcatgtt tgtgttagat ccgtgtttgt 6180

-continued

gttagatccg tgctgctagc gttcgtacac ggatgcgacc tgtacgtcag acacgttctg 6240

attgctaact tgccagtgtt tctctttggg gaatcctggg atggctctag ccgttccgca 6300

gacgggatcg atttcatgat ttttttttgtt tcgttgcata gggtttggtt tgccctttc 6360

ctttatttca atatatgccg tgcacttgtt tgtcgggtca tcttttcatg ctttttttg 6420

tcttggttgt gatgatgtgg tctggttggg cggtcgttct agatcggagt agaattctgt 6480

ttcaaactac ctggtggatt tattaatttt ggatctgtat gtgtgtgcca tacatattca 6540

tagttacgaa ttgaagatga tggatggaaa tatcgatcta ggataggtat acatgttgat 6600

gcgggtttta ctgatgcata tacagagatg ctttttgttc gcttggttgt gatgatgtgg 6660

tgtggttggg cggtcgttca ttcgttctag atcggagtag aatactgttt caaactacct 6720

ggtgtattta ttaattttgg aactgtatgt gtgtgtcata catcttcata gttacgagtt 6780

taagatggat ggaaatatcg atctaggata ggtatacatg ttgatgtggg ttttactgat 6840

gcatatacat gatggcatat gcagcatcta ttcatatgct ctaaccttga gtacctatct 6900

attataataa acaagtatgt tttataatta ttttgatctt gatatacttg gatgatggca 6960

tatgcagcag ctatatgtgg attttttttag ccctgccttc atacgctatt tatttgcttg 7020

gtactgtttc ttttgtcgat gctcaccctg ttgtttggtg ttacttctgc aggtcgactt 7080

taacttagcc taggatccac acgacaccat gtcccccgag cgccgccccg tcgagatccg 7140

cccggccacc gccgccgaca tggccgccgt gtgcgacatc gtgaaccact acatcgagac 7200

ctccaccgtg aacttccgca ccgagccgca gaccccgcag gagtggatcg acgacctgga 7260

gcgcctccag gaccgctacc cgtggctcgt ggccgaggtg gagggcgtgg tggccggcat 7320

cgcctacgcc ggcccgtgga aggcccgcaa cgcctacgac tggaccgtgg agtccaccgt 7380

gtacgtgtcc caccgccacc agcgcctcgg cctcggctcc accctctaca cccacctcct 7440

caagagcatg gaggcccagg gcttcaagtc cgtggtggcc gtgatcggcc tcccgaacga 7500

cccgtccgtg cgcctccacg aggccctcgg ctacaccgcc cgcggcaccc tccgcgccgc 7560

cggctacaag cacggcggct ggcacgacgt cggcttctgg cagcgcgact tcgagctgcc 7620

ggccccgccg cgcccggtgc gcccggtgac gcagatctga gtcgaaacct agacttgtcc 7680

atcttctgga ttggccaact taattaatgt atgaaataaa aggatgcaca catagtgaca 7740

tgctaatcac tataatgtgg gcatcaaagt tgtgtgttat gtgtaattac tagttatctg 7800

aataaaagag aaagagatca tccatatttc ttatcctaaa tgaatgtcac gtgtctttat 7860

aattctttga tgaaccagat gcatttcatt aaccaaatcc atatacatat aaatattaat 7920

catatataat taatatcaat tgggttagca aaacaaatct agtctaggtg tgttttgcga 7980

attgcggccg ctctagcgta tacgaagttc ctattccgaa gttcctattc tctagaaagt 8040

ataggaactt ctgattccga tgacttcgta ggttcctagc tcaagccgct cgtgtccaag 8100

cgtcacttac gattagctaa tgattacggc atctaggacc gactagtaag tgactagggt 8160

cacgtgaccc tagtcactta tacgtagaat taattcattc cgattaatcg tggcctcttg 8220

ctcttcagga tgaagagcta tgtttaaacg tgcaagcgct actagacaat tcagtacatt 8280

aaaaacgtcc gcaatgtgtt attaagttgt ctaagcgtca atttgtttac accacaatat 8340

atcctgccac cagccagcca acagctcccc gaccggcagc tcggcacaaa atcaccactc 8400

gatacaggca gcccatcagt ccgggacggc gtcagcggga gagccgttgt aaggcggcag 8460

-continued

```
actttgctca tgttaccgat gctattcgga agaacggcaa ctaagctgcc gggtttgaaa   8520
cacggatgat ctcgcggagg gtagcatgtt gattgtaacg atgacagagc gttgctgcct   8580
gtgatcaaat atcatctccc tcgcagagat ccgaattatc agccttctta ttcatttctc   8640
gcttaaccgt gacaggctgt cgatcttgag aactatgccg acataatagg aaatcgctgg   8700
ataaagccgc tgaggaagct gagtggcgct atttctttag aagtgaacgt tgacgatcgt   8760
cgaccgtacc ccgatgaatt aattcggacg tacgttctga acacagctgg atacttactt   8820
gggcgattgt catacatgac atcaacaatg tacccgtttg tgtaaccgtc tcttggaggt   8880
tcgtatgaca ctagtggttc ccctcagctt gcgactagat gttgaggcct aacattttat   8940
tagagagcag gctagttgct tagatacatg atcttcaggc cgttatctgt cagggcaagc   9000
gaaaattggc catttatgac gaccaatgcc ccgcagaagc tcccatcttt gccgccatag   9060
acgccgcgcc ccccttttgg ggtgtagaac atccttttgc cagatgtgga aaagaagttc   9120
gttgtcccat tgttggcaat gacgtagtag ccggcgaaag tgcgagaccc atttgcgcta   9180
tatataagcc tacgatttcc gttgcgacta ttgtcgtaat tggatgaact attatcgtag   9240
ttgctctcag agttgtcgta atttgatgga ctattgtcgt aattgcttat ggagttgtcg   9300
tagttgcttg gagaaatgtc gtagttggat ggggagtagt catagggaag acgagcttca   9360
tccactaaaa caattggcag gtcagcaagt gcctgccccg atgccatcgc aagtacgagg   9420
cttagaacca ccttcaacag atcgcgcata gtcttcccca gctctctaac gcttgagtta   9480
agccgcgccg cgaagcggcg tcggcttgaa cgaattgtta gacattattt gccgactacc   9540
ttggtgatct cgcctttcac gtagtgaaca aattcttcca actgatctgc gcgcgaggcc   9600
aagcgatctt cttgtccaag ataagcctgc ctagcttcaa gtatgacggg ctgatactgg   9660
gccggcaggc gctccattgc ccagtcggca gcgacatcct tcggcgcgat tttgccggtt   9720
actgcgctgt accaaatgcg ggacaacgta agcactacat ttcgctcatc gccagcccag   9780
tcgggcggcg agttccatag cgttaaggtt tcatttagcg cctcaaatag atcctgttca   9840
ggaaccggat caaagagttc ctccgccgct ggacctacca aggcaacgct atgttctctt   9900
gcttttgtca gcaagatagc cagatcaatg tcgatcgtgg ctggctcgaa gatacctgca   9960
agaatgtcat tgcgctgcca ttctccaaat tgcagttcgc gcttagctgg ataacgccac  10020
ggaatgatgt cgtcgtgcac aacaatggtg acttctacag cgcggagaat ctcgctctct  10080
ccaggggaag ccgaagtttc caaaaggtcg ttgatcaaag ctcgccgcgt tgtttcatca  10140
agccttacag tcaccgtaac cagcaaatca atatcactgt gtggcttcag gccgccatcc  10200
actgcggagc cgtacaaatg tacggccagc aacgtcggtt cgagatggcg ctcgatgacg  10260
ccaactacct ctgatagttg agtcgatact tcggcgatca ccgcttccct catgatgttt  10320
aactcctgaa ttaagccgcg ccgcgaagcg gtgtcggctt gaatgaattg ttaggcgtca  10380
tcctgtgctc ccgagaacca gtaccagtac atcgctgttt cgttcgagac ttgaggtcta  10440
gttttatacg tgaacaggtc aatgccgccg agagtaaagc cacattttgc gtacaaattg  10500
caggcaggta cattgttcgt ttgtgtctct aatcgtatgc caaggagctg tctgcttagt  10560
gcccactttt tcgcaaattc gatgagactg tgcgcgactc ctttgcctcg gtgcgtgtgc  10620
gacacaacaa tgtgttcgat agaggctaga tcgttccatg ttgagttgag ttcaatcttc  10680
ccgacaagct cttggtcgat gaatgcgcca tagcaagcag agtcttcatc agagtcatca  10740
```

-continued

tccgagatgt aatccttccg gtaggggctc acacttctgg tagatagttc aaagccttgg 10800

tcggataggt gcacatcgaa cacttcacga acaatgaaat ggttctcagc atccaatgtt 10860

tccgccacct gctcagggat caccgaaatc ttcatatgac gcctaacgcc tggcacagcg 10920

gatcgcaaac ctggcgcggc ttttggcaca aaaggcgtga caggtttgcg aatccgttgc 10980

tgccacttgt taacccttt gccagatttg gtaactataa tttatgttag aggcgaagtc 11040

ttgggtaaaa actggcctaa aattgctggg gatttcagga aagtaaacat caccttccgg 11100

ctcgatgtct attgtagata tatgtagtgt atctacttga tcgggggatc tgctgcctcg 11160

cgcgtttcgg tgatgacggt gaaaacctct gacacatgca gctcccggag acggtcacag 11220

cttgtctgta agcggatgcc gggagcagac aagcccgtca gggcgcgtca gcgggtgttg 11280

gcgggtgtcg gggcgcagcc atgacccagt cacgtagcga tagcggagtg tatactggct 11340

taactatgcg gcatcagagc agattgtact gagagtgcac catatgcggt gtgaaatacc 11400

gcacagatgc gtaaggagaa aataccgcat caggcgctct tccgcttcct cgctcactga 11460

ctcgctgcgc tcggtcgttc ggctgcggcg agcggtatca gctcactcaa aggcggtaat 11520

acggttatcc acagaatcag gggataacgc aggaaagaac atgtgagcaa aaggccagca 11580

aaaggccagg aaccgtaaaa aggccgcgtt gctggcgttt ttccataggc tccgcccccc 11640

tgacgagcat cacaaaaatc gacgctcaag tcagaggtgg cgaaacccga caggactata 11700

aagataccag gcgtttcccc ctggaagctc cctcgtgcgc tctcctgttc cgaccctgcc 11760

gcttaccgga tacctgtccg cctttctccc ttcgggaagc gtggcgcttt ctcatagctc 11820

acgctgtagg tatctcagtt cggtgtaggt cgttcgctcc aagctgggct gtgtgcacga 11880

acccccgtt cagcccgacc gctgcgcctt atccggtaac tatcgtcttg agtccaaccc 11940

ggtaagacac gacttatcgc cactggcagc agccactggt aacaggatta gcagagcgag 12000

gtatgtaggc ggtgctacag agttcttgaa gtggtggcct aactacggct acactagaag 12060

gacagtattt ggtatctgcg ctctgctgaa gccagttacc ttcggaaaaa gagttggtag 12120

ctcttgatcc ggcaaacaaa ccaccgctgg tagcggtggt ttttttgttt gcaagcagca 12180

gattacgcgc agaaaaaaag gatctcaaga agatcctttg atcttttcta cggggtctga 12240

cgctcagtgg aacgaaaact cacgttaagg gattttggtc atgagattat caaaaaggat 12300

cttcacctag atccttttaa attaaaaatg aagttttaaa tcaatctaaa gtatatatga 12360

gtaaacttgg tctgacagtt accaatgctt aatcagtgag gcacctatct cagcgatctg 12420

tctatttcgt tcatccatag ttgcctgact ccccgtcgtg tagataacta cgatacggga 12480

gggcttacca tctggcccca gtgctgcaat gataccgcga gacccacgct caccggctcc 12540

agatttatca gcaataaacc agccagccgg aagggccgag cgcagaagtg gtcctgcaac 12600

tttatccgcc tccatccagt ctattaattg ttgccgggaa gctagagtaa gtagttcgcc 12660

agttaatagt ttgcgcaacg ttgttgccat tgctgcaggg gggggggggg gggggactt 12720

ccattgttca ttccacggac aaaaacagag aaaggaaacg acagaggcca aaaagcctcg 12780

ctttcagcac ctgtcgtttc ctttcttttc agagggtatt ttaaataaaa acattaagtt 12840

atgacgaaga agaacggaaa cgccttaaac cggaaaattt tcataaatag cgaaaacccg 12900

cgaggtcgcc gccccgtaac acctgtcgga tcaccggaaa ggacccgtaa agtgataatg 12960

attatcatct acatatcaca acgtgcgtgg aggccatcaa accacgtcaa ataatcaatt 13020

-continued

atgacgcagg tatcgtatta attgatctgc atcaacttaa cgtaaaaaca acttcagaca 13080

atacaaatca gcgacactga atacggggca acctcatgtc ccccccccc ccccccctgc 13140

aggcatcgtg gtgtcacgct cgtcgtttgg tatggcttca ttcagctccg gttcccaacg 13200

atcaaggcga gttacatgat cccccatgtt gtgcaaaaaa gcggttagct ccttcggtcc 13260

tccgatcgtt gtcagaagta agttggccgc agtgttatca ctcatggtta tggcagcact 13320

gcataattct cttactgtca tgccatccgt aagatgcttt tctgtgactg gtgagtactc 13380

aaccaagtca ttctgagaat agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaac 13440

acgggataat accgcgccac atagcagaac tttaaaagtg ctcatcattg gaaaacgttc 13500

ttcggggcga aaactctcaa ggatcttacc gctgttgaga tccagttcga tgtaacccac 13560

tcgtgcaccc aactgatctt cagcatcttt tactttcacc agcgtttctg ggtgagcaaa 13620

aacaggaagg caaaatgccg caaaaaaggg aataagggcg acacggaaat gttgaatact 13680

catactcttc ctttttcaat attattgaag catttatcag ggttattgtc tcatgagcgg 13740

atacatattt gaatgtattt agaaaaataa acaaataggg gttccgcgca catttccccg 13800

aaaagtgcca cctgacgtct aagaaaccat tattatcatg acattaacct ataaaaatag 13860

gcgtatcacg aggccctttc gtcttcaaga attcggagct tttgccattc tcaccggatt 13920

cagtcgtcac tcatggtgat ttctcacttg ataaccttat ttttgacgag gggaaattaa 13980

taggttgtat tgatgttgga cgagtcggaa tcgcagaccg ataccaggat cttgccatcc 14040

tatggaactg cctcggtgag ttttctcctt cattacagaa acggcttttt caaaaatatg 14100

gtattgataa tcctgatatg aataaattgc agtttcattt gatgctcgat gagtttttct 14160

aatcagaatt ggttaattgg ttgtaacact ggcagagcat tacgctgact tgacgggacg 14220

gcggctttgt tgaataaatc gaacttttgc tgagttgaag gatcagatca cgcatcttcc 14280

cgacaacgca gaccgttccg tggcaaagca aaagttcaaa atcaccaact ggtccaccta 14340

caacaaagct ctcatcaacc gtggctccct cactttctgg ctggatgatg gggcgattca 14400

ggcctggtat gagtcagcaa caccttcttc acgaggcaga cctcagcgcc agaaggccgc 14460

cagagaggcc gagcgcggcc gtgaggcttg gacgctaggg cagggcatga aaaagcccgt 14520

agcgggctgc tacgggcgtc tgacgcggtg gaaaggggga ggggatgttg tctacatggc 14580

tctgctgtag tgagtgggtt gcgctccggc agcggtcctg atcaatcgtc acccttttctc 14640

ggtccttcaa cgttcctgac aacgagcctc cttttcgcca atccatcgac aatcaccgcg 14700

agtccctgct cgaacgctgc gtccggaccg gcttcgtcga aggcgtctat cgcggcccgc 14760

aacagcggcg agagcggagc ctgttcaacg gtgccgccgc gctcgccggc atcgctgtcg 14820

ccggcctgct cctcaagcac ggccccaaca gtgaagtagc tgattgtcat cagcgcattg 14880

acggcgtccc cggccgaaaa acccgcctcg cagaggaagc gaagctgcgc gtcggccgtt 14940

tccatctgcg gtgcgcccgg tcgcgtgccg gcatggatgc gcgcgccatc gcggtaggcg 15000

agcagcgcct gcctgaagct gcgggcattc ccgatcagaa atgagcgcca gtcgtcgtcg 15060

gctctcggca ccgaatgcgt atgattctcc gccagcatgg cttcggccag tgcgtcgagc 15120

agcgcccgct tgttcctgaa gtgccagtaa agcgccggct gctgaacccc caaccgttcc 15180

gccagtttgc gtgtcgtcag accgtctacg ccgacctcgt tcaacaggtc cagggcggca 15240

cggatcactg tattcggctg caactttgtc atgcttgaca ctttatcact gataaacata 15300

-continued

atatgtccac caacttatca gtgataaaga atccgcgcgt tcaatcggac cagcggaggc 15360

tggtccggag gccagacgtg aaacccaaca tacccctgat cgtaattctg agcactgtcg 15420

cgctcgacgc tgtcggcatc ggcctgatta tgccggtgct gccgggcctc ctgcgcgatc 15480

tggttcactc gaacgacgtc accgcccact atggcattct gctggcgctg tatgcgttgg 15540

tgcaatttgc ctgcgcacct gtgctgggcg cgctgtcgga tcgtttcggg cggcggccaa 15600

tcttgctcgt ctcgctggcc ggcgccactg tcgactacgc catcatggcg acagcgcctt 15660

tcctttgggt tctctatatc gggcggatcg tggccggcat caccggggcg actggggcgg 15720

tagccggcgc ttatattgcc gatatcactg atggcgatga gcgcgcgcgg cacttcggct 15780

tcatgagcgc ctgtttcggg ttcgggatgg tcgcgggacc tgtgctcggt gggctgatgg 15840

gcggtttctc cccccacgct ccgttcttcg ccgcggcagc cttgaacggc ctcaatttcc 15900

tgacgggctg tttccttttg ccggagtcgc acaaaggcga acgccggccg ttacgccggg 15960

aggctctcaa cccgctcgct tcgttccggt gggcccgggg catgaccgtc gtcgccgccc 16020

tgatggcggt cttcttcatc atgcaacttg tcggacaggt gccggccgcg ctttgggtca 16080

ttttcggcga ggatcgcttt cactgggacg cgaccacgat cggcatttcg cttgccgcat 16140

ttggcattct gcattcactc gcccaggcaa tgatcaccgg ccctgtagcc gcccggctcg 16200

gcgaaaggcg ggcactcatg ctcggaatga ttgccgacgg cacaggctac atcctgcttg 16260

ccttcgcgac acggggatgg atggcgttcc cgatcatggt cctgcttgct tcgggtggca 16320

tcggaatgcc ggcgctgcaa gcaatgttgt ccaggcaggt ggatgaggaa cgtcaggggc 16380

agctgcaagg ctcactggcg gcgctcacca gcctgacctc gatcgtcgga cccctcctct 16440

tcacggcgat ctatgcggct tctataacaa cgtggaacgg gtgggcatgg attgcaggcg 16500

ctgccctcta cttgctctgc ctgccggcgc tgcgtcgcgg gctttggagc ggcgcagggc 16560

aacgagccga tcgctgatcg tggaaacgat aggcctatgc catgcgggtc aaggcgactt 16620

ccggcaagct atacgcgccc taggagtgcg gttggaacgt tggcccagcc agatactccc 16680

gatcacgagc aggacgccga tgatttgaag cgcactcagc gtctgatcca agaacaacca 16740

tcctagcaac acggcggtcc ccgggctgag aaagcccagt aaggaaacaa ctgtaggttc 16800

gagtcgcgag atcccccgga accaaaggaa gtaggttaaa cccgctccga tcaggccgag 16860

ccacgccagg ccgagaacat tggttcctgt aggcatcggg attggcggat caaacactaa 16920

agctactgga acgagcagaa gtcctccggc cgccagttgc caggcggtaa aggtgagcag 16980

aggcacggga ggttgccact tgcgggtcag cacggttccg aacgccatgg aaaccgcccc 17040

cgccaggccc gctgcgacgc cgacaggatc tagcgctgcg tttggtgtca acaccaacag 17100

cgccacgccc gcagttccgc aaatagcccc caggaccgcc atcaatcgta tcgggctacc 17160

tagcagagcg gcagagatga acacgaccat cagcggctgc acagcgccta ccgtcgccgc 17220

gaccccgccc ggcaggcggt agaccgaaat aaacaacaag ctccagaata gcgaaatatt 17280

aagtgcgccg aggatgaaga tgcgcatcca ccagattccc gttggaatct gtcggacgat 17340

catcacgagc aataaacccg ccggcaacgc ccgcagcagc ataccggcga cccctcggcc 17400

tcgctgttcg ggctccacga aaacgccgga cagatgcgcc ttgtgagcgt ccttggggcc 17460

gtcctcctgt ttgaagaccg acagcccaat gatctcgccg tcgatgtagg cgccgaatgc 17520

cacggcatct cgcaaccgtt cagcgaacgc ctccatgggc tttttctcct cgtgctcgta 17580

-continued

aacggacccg aacatctctg gagctttctt cagggccgac aatcggatct cgcggaaatc 17640

ctgcacgtcg gccgctccaa gccgtcgaat ctgagcctta atcacaattg tcaattttaa 17700

tcctctgttt atcggcagtt cgtagagcgc gccgtgcgtc ccgagcgata ctgagcgaag 17760

caagtgcgtc gagcagtgcc cgcttgttcc tgaaatgcca gtaaagcgct ggctgctgaa 17820

cccccagccg gaactgaccc cacaaggccc tagcgtttgc aatgcaccag gtcatcattg 17880

acccaggcgt gttccaccag gccgctgcct cgcaactctt cgcaggcttc gccgacctgc 17940

tcgcgccact tcttcacgcg ggtggaatcc gatccgcaca tgaggcggaa ggtttccagc 18000

ttgagcgggt acggctcccg gtgcgagctg aaatagtcga acatccgtcg ggccgtcggc 18060

gacagcttgc ggtacttctc ccatatgaat ttcgtgtagt ggtcgccagc aaacagcacg 18120

acgatttcct cgtcgatcag gacctggcaa cgggacgttt tcttgccacg gtccaggacg 18180

cggaagcggt gcagcagcga caccgattcc aggtgcccaa cgcggtcgga cgtgaagccc 18240

atcgccgtcg cctgtaggcg cgacaggcat tcctcggcct tcgtgtaata ccggccattg 18300

atcgaccagc ccaggtcctg gcaaagctcg tagaacgtga aggtgatcgg ctcgccgata 18360

ggggtgcgct tcgcgtactc caacacctgc tgccacacca gttcgtcatc gtcggcccgc 18420

agctcgacgc cggtgtaggt gatcttcacg tccttgttga cgtggaaaat gaccttgttt 18480

tgcagcgcct cgcgcgggat tttcttgttg cgcgtggtga acagggcaga gcgggccgtg 18540

tcgtttggca tcgctcgcat cgtgtccggc cacggcgcaa tatcgaacaa ggaaagctgc 18600

atttccttga tctgctgctt cgtgtgtttc agcaacgcgg cctgcttggc ctcgctgacc 18660

tgttttgcca ggtcctcgcc ggcggttttt cgcttcttgg tcgtcatagt tcctcgcgtg 18720

tcgatggtca tcgacttcgc caaacctgcc gcctcctgtt cgagacgacg cgaacgctcc 18780

acggcggccg atggcgcggg cagggcaggg ggagccagtt gcacgctgtc gcgctcgatc 18840

ttggccgtag cttgctggac catcgagccg acggactgga aggtttcgcg gggcgcacgc 18900

atgacggtgc ggcttgcgat ggtttcggca tcctcggcgg aaaaccccgc gtcgatcagt 18960

tcttgcctgt atgccttccg gtcaaacgtc cgattcattc accctccttg cgggattgcc 19020

ccgactcacg ccggggcaat gtgcccttat tcctgatttg acccgcctgg tgccttggtg 19080

tccagataat ccaccttatc ggcaatgaag tcggtcccgt agaccgtctg gccgtccttc 19140

tcgtacttgg tattccgaat cttgccctgc acgaatacca gcgacccctt gcccaaatac 19200

ttgccgtggg cctcggcctg agagccaaaa cacttgatgc ggaagaagtc ggtgcgctcc 19260

tgcttgtcgc cggcatcgtt gcgccactct tcattaaccg ctatatcgaa aattgcttgc 19320

ggcttgttag aattgccatg acgtacctcg gtgtcacggg taagattacc gataaactgg 19380

aactgattat ggctcatatc gaaagtctcc ttgagaaagg agactctagt ttagctaaac 19440

attggttccg ctgtcaagaa ctttagcggc taaaattttg cgggccgcga ccaaaggtgc 19500

gaggggcggc ttccgctgtg tacaaccaga tatttttcac caacatcctt cgtctgctcg 19560

atgagcgggg catgacgaaa catgagctgt cggagagggc aggggtttca atttcgtttt 19620

tatcagactt aaccaacggt aaggccaacc cctcgttgaa ggtgatggag gccattgccg 19680

acgccctgga aactccccta cctcttctcc tggagtccac cgaccttgac cgcgaggcac 19740

tcgcggagat tgcgggtcat cctttcaaga gcagcgtgcc gcccggatac gaacgcatca 19800

gtgtggtttt gccgtcacat aaggcgttta tcgtaaagaa atggggcgac gacacccgaa 19860

-continued

aaaagctgcg tggaaggctc tgacgccaag ggttagggct tgcacttcct tctttagccg 19920

ctaaaacggc cccttctctg cgggccgtcg gctcgcgcat catatcgaca tcctcaacgg 19980

aagccgtgcc gcgaatggca tcgggcgggt gcgctttgac agttgttttc tatcagaacc 20040

cctacgtcgt gcggttcgat tagctgtttg tcttgcaggc taaacacttt cggtatatcg 20100

tttgcctgtg cgataatgtt gctaatgatt tgttgcgtag gggttactga aaagtgagcg 20160

ggaaagaaga gtttcagacc atcaaggagc gggccaagcg caagctggaa cgcgacatgg 20220

gtgcggacct gttggccgcg ctcaacgacc cgaaaaccgt tgaagtcatg ctcaacgcgg 20280

acggcaaggt gtggcacgaa cgccttggcg agccgatgcg gtacatctgc gacatgcggc 20340

ccagccagtc gcaggcgatt atagaaacgg tggccggatt ccacggcaaa gaggtcacgc 20400

ggcattcgcc catcctggaa ggcgagttcc ccttggatgg cagccgcttt gccggccaat 20460

tgccgccggt cgtggccgcg ccaacctttg cgatccgcaa gcgcgcggtc gccatcttca 20520

cgctggaaca gtacgtcgag gcgggcatca tgacccgcga gcaatacgag gtcattaaaa 20580

gcgccgtcgc ggcgcatcga aacatcctcg tcattggcgg tactggctcg ggcaagacca 20640

cgctcgtcaa cgcgatcatc aatgaaatgg tcgccttcaa cccgtctgag cgcgtcgtca 20700

tcatcgagga caccggcgaa atccagtgcg ccgcagagaa cgccgtccaa taccacacca 20760

gcatcgacgt ctcgatgacg ctgctgctca agacaacgct gcgtatgcgc cccgaccgca 20820

tcctggtcgg tgaggtacgt ggccccgaag cccttgatct gttgatggcc tggaacaccg 20880

ggcatgaagg aggtgccgcc accctgcacg caaacaaccc caaagcgggc ctgagccggc 20940

tcgccatgct tatcagcatg cacccggatt caccgaaacc cattgagccg ctgattggcg 21000

aggcggttca tgtggtcgtc catatcgcca ggacccctag cggccgtcga gtgcaagaaa 21060

ttctcgaagt tcttggttac gagaacggcc agtacatcac caaaaccctg taaggagtat 21120

ttccaatgac aacggctgtt ccgttccgtc tgaccatgaa tcgcggcatt ttgttctacc 21180

ttgccgtgtt cttcgttctc gctctcgcgt tatccgcgca tccggcgatg gcctcggaag 21240

gcaccggcgg cagcttgcca tatgagagct ggctgacgaa cctgcgcaac tccgtaaccg 21300

gcccggtggc cttcgcgctg tccatcatcg gcatcgtcgt cgccggcggc gtgctgatct 21360

tcggcggcga actcaacgcc ttcttccgaa ccctgatctt cctggttctg gtgatggcgc 21420

tgctggtcgg cgcgcagaac gtgatgagca ccttcttcgg tcgtggtgcc gaaatcgcgg 21480

ccctcggcaa cggggcgctg caccaggtgc aagtcgcggc ggcggatgcc gtgcgtgcgg 21540

tagcggctgg acggctcgcc taatcatggc tctgcgcacg atccccatcc gtcgcgcagg 21600

caaccgagaa aacctgttca tgggtggtga tcgtgaactg gtgatgttct cgggcctgat 21660

ggcgtttgcg ctgattttca gcgcccaaga gctgcgggcc accgtggtcg gtctgatcct 21720

gtggttcggg gcgctctatg cgttccgaat catggcgaag gccgatccga agatgcggtt 21780

cgtgtacctg cgtcaccgcc ggtacaagcc gtattacccg gcccgctcga ccccgttccg 21840

cgagaacacc aatagccaag ggaagcaata ccgatgatcc aagcaattgc gattgcaatc 21900

gcgggcctcg gcgcgcttct gttgttcatc ctctttgccc gcatccgcgc ggtcgatgcc 21960

gaactgaaac tgaaaaagca tcgttccaag gacgccggcc tggccgatct gctcaactac 22020

gccgctgtcg tcgatgacgg cgtaatcgtg ggcaagaacg gcagctttat ggctgcctgg 22080

ctgtacaagg gcgatgacaa cgcaagcagc accgaccagc agcgcgaagt agtgtccgcc 22140

-continued

cgcatcaacc aggccctcgc gggcctggga agtgggtgga tgatccatgt ggacgccgtg 22200

cggcgtcctg ctccgaacta cgcggagcgg ggcctgtcgg cgttccctga ccgtctgacg 22260

gcagcgattg aagaagagcg ctcggtcttg ccttgctcgt cggtgatgta cttcaccagc 22320

tccgcgaagt cgctcttctt gatggagcgc atggggacgt gcttggcaat cacgcgcacc 22380

ccccggccgt tttagcggct aaaaaagtca tggctctgcc ctcgggcgga ccacgcccat 22440

catgaccttg ccaagctcgt cctgcttctc ttcgatcttc gccagcaggg cgaggatcgt 22500

ggcatcaccg aaccgcgccg tgcgcgggtc gtcggtgagc cagagtttca gcaggccgcc 22560

caggcggccc aggtcgccat tgatgcgggc cagctcgcgg acgtgctcat agtccacgac 22620

gcccgtgatt ttgtagccct ggccgacggc cagcaggtag gccgacaggc tcatgccggc 22680

cgccgccgcc ttttcctcaa tcgctcttcg ttcgtctgga aggcagtaca ccttgatagg 22740

tgggctgccc ttcctggttg gcttggtttc atcagccatc cgcttgccct catctgttac 22800

gccggcggta gccggccagc ctcgcagagc aggattcccg ttgagcaccg ccaggtgcga 22860

ataagggaca gtgaagaagg aacacccgct cgcgggtggg cctacttcac ctatcctgcc 22920

cggctgacgc cgttggatac accaaggaaa gtctacacga acccctttggc aaaatcctgt 22980

atatcgtgcg aaaaaggatg gatataccga aaaaatcgct ataatgaccc cgaagcaggg 23040

ttatgcagcg gaaaagcgct gcttccctgc tgttttgtgg aatatctacc gactggaaac 23100

aggcaaatgc aggaaattac tgaactgagg ggacaggcga gagacgatgc caaagagcta 23160

caccgacgag ctggccgagt gggttgaatc ccgcgcggcc aagaagcgcc ggcgtgatga 23220

ggctgcggtt gcgttcctgg cggtgagggc ggatgtcgag gcggcgttag cgtccggcta 23280

tgcgctcgtc accatttggg agcacatgcg ggaaacgggg aaggtcaagt tctcctacga 23340

gacgttccgc tcgcacgcca ggcggcacat caaggccaag cccgccgatg tgcccgcacc 23400

gcaggccaag gctgcggaac ccgcgccggc acccaagacg ccggagccac ggcggccgaa 23460

gcaggggggc aaggctgaaa agccggcccc cgctgcggcc ccgaccggct tcaccttcaa 23520

cccaacaccg gacaaaaagg atctactgta atggcgaaaa ttcacatggt tttgcagggc 23580

aagggcgggg tcggcaagtc ggccatcgcc gcgatcattg cgcagtacaa gatggacaag 23640

gggcagacac ccttgtgcat cgacaccgac ccggtgaacg cgacgttcga gggctacaag 23700

gccctgaacg tccgccggct gaacatcatg gccggcgacg aaattaactc gcgcaacttc 23760

gacaccctgg tcgagctgat tgcgccgacc aaggatgacg tggtgatcga caacggtgcc 23820

agctcgttcg tgcctctgtc gcattacctc atcagcaacc aggtgccggc tctgctgcaa 23880

gaaatggggc atgagctggt catccatacc gtcgtcaccg gcggccaggc tctcctggac 23940

acggtgagcg gcttcgccca gctcgccagc cagttcccgg ccgaagcgct tttcgtggtc 24000

tggctgaacc cgtattgggg gcctatcgag catgagggca agagctttga gcagatgaag 24060

gcgtacacgg ccaacaaggc ccgcgtgtcg tccatcatcc agattccggc cctcaaggaa 24120

gaaacctacg gccgcgattt cagcgacatg ctgcaagagc ggctgacgtt cgaccaggcg 24180

ctggccgatg aatcgctcac gatcatgacg cggcaacgcc tcaagatcgt gcggcgcggc 24240

ctgtttgaac agctcgacgc ggcggccgtg ctatgagcga ccagattgaa gagctgatcc 24300

gggagattgc ggccaagcac ggcatcgccg tcggccgcga cgacccggtg ctgatcctgc 24360

ataccatcaa cgcccggctc atggccgaca gtgcggccaa gcaagaggaa atccttgccg 24420

-continued

cgttcaagga agagctggaa gggatcgccc atcgttgggg cgaggacgcc aaggccaaag 24480

cggagcggat gctgaacgcg gccctggcgg ccagcaagga cgcaatggcg aaggtaatga 24540

aggacagcgc cgcgcaggcg gccgaagcga tccgcaggga aatcgacgac ggccttggcc 24600

gccagctcgc ggccaaggtc gcggacgcgc ggcgcgtggc gatgatgaac atgatcgccg 24660

gcggcatggt gttgttcgcg gccgccctgg tggtgtgggc ctcgttatga atcgcagagg 24720

cgcagatgaa aaagcccggc gttgccgggc tttgtttttg cgttagctgg gcttgtttga 24780

caggcccaag ctctgactgc gcccgcgctc gcgctcctgg gcctgtttct tctcctgctc 24840

ctgcttgcgc atcagggcct ggtgccgtcg ggctgcttca cgcatcgaat cccagtcgcc 24900

ggccagctcg ggatgctccg cgcgcatctt gcgcgtcgcc agttcctcga tcttgggcgc 24960

gtgaatgccc atgccttcct tgatttcgcg caccatgtcc agccgcgtgt gcagggtctg 25020

caagcgggct tgctgttggg cctgctgctg ctgccaggcg gcctttgtac gcggcaggga 25080

cagcaagccg ggggcattgg actgtagctg ctgcaaacgc gcctgctgac ggtctacgag 25140

ctgttctagg cggtcctcga tgcgctccac ctggtcatgc tttgcctgca cgtagagcgc 25200

aagggtctgc tggtaggtct gctcgatggg cgcggattct aagagggcct gctgttccgt 25260

ctcggcctcc tgggccgcct gtagcaaatc ctcgccgctg ttgccgctgg actgctttac 25320

tgccggggac tgctgttgcc ctgctcgcgc cgtcgtcgca gttcggcttg cccccactcg 25380

attgactgct tcatttcgag ccgcagcgat gcgatctcgg attgcgtcaa cggacggggc 25440

agcgcggagg tgtccggctt ctccttgggt gagtcggtcg atgccatagc caaaggtttc 25500

cttccaaaat gcgtccattg ctggaccgtg tttctcattg atgcccgcaa gcatcttcgg 25560

cttgaccgcc aggtcaagcg cgccttcatg ggcggtcatg acggacgccg ccatgacctt 25620

gccgccgttg ttctcgatgt agccgcgtaa tgaggcaatg gtgccgccca tcgtcagcgt 25680

gtcatcgaca acgatgtact tctggccggg gatcacctcc ccctcgaaag tcgggttgaa 25740

cgccaggcga tgatctgaac cggctccggt tcgggcgacc ttctcccgct gcacaatgtc 25800

cgtttcgacc tcaaggccaa ggcggtcggc cagaacgacc gccatcatgg ccggaatctt 25860

gttgttcccc gccgcctcga cggcgaggac tggaacgatg cggggcttgt cgtcgccgat 25920

cagcgtcttg agctgggcaa cagtgtcgtc cgaaatcagg cgctcgacca aattaagcgc 25980

cgcttccgcg tcgccctgct tcgcagcctg gtattcaggc tcgttggtca aagaaccaag 26040

gtcgccgttg cgaaccacct tcgggaagtc tccccacggt gcgcgctcgg ctctgctgta 26100

gctgctcaag acgcctccct ttttagccgc taaaactcta acgagtgcgc ccgcgactca 26160

acttgacgct ttcggcactt acctgtgcct tgccacttgc gtcataggtg atgcttttcg 26220

cactcccgat ttcaggtact ttatcgaaat ctgaccgggc gtgcattaca aagttcttcc 26280

ccacctgttg gtaaatgctg ccgctatctg cgtggacgat gctgccgtcg tggcgctgcg 26340

acttatcggc cttttgggcc atatagatgt tgtaaatgcc aggtttcagg gccccggctt 26400

tatctacctt ctggttcgtc catgcgcctt ggttctcggt ctggacaatt ctttgcccat 26460

tcatgaccag gaggcggtgt ttcattgggt gactcctgac ggttgcctct ggtgttaaac 26520

gtgtcctggt cgcttgccgg ctaaaaaaaa gccgacctcg gcagttcgag gccggctttc 26580

cctagagccg ggcgcgtcaa ggttgttcca tctattttag tgaactgcgt tcgatttatc 26640

agttactttc ctcccgcttt gtgtttcctc ccactcgttt ccgcgtctag ccgacccctc 26700

-continued

aacatagcgg cctcttcttg ggctgccttt gcctcttgcc gcgcttcgtc acgctcggct 26760

tgcaccgtcg taaagcgctc ggcctgcctg gccgcctctt gcgccgccaa cttcctttgc 26820

tcctggtggg cctcggcgtc ggcctgcgcc ttcgctttca ccgctgccaa ctccgtgcgc 26880

aaactctccg cttcgcgcct ggtggcgtcg cgctcgccgc gaagcgcctg catttcctgg 26940

ttggccgcgt ccagggtctt gcggctctct tctttgaatg cgcgggcgtc ctggtgagcg 27000

tagtccagct cggcgcgcag ctcctgcgct cgacgctcca cctcgtcggc ccgctgcgtc 27060

gccagcgcgg cccgctgctc ggctcctgcc agggcggtgc gtgcttcggc cagggcttgc 27120

cgctggcgtg cggccagctc ggccgcctcg gcggcctgct gctctagcaa tgtaacgcgc 27180

gcctgggctt cttccagctc gcgggcctgc gcctcgaagg cgtcggccag ctccccgcgc 27240

acggcttcca actcgttgcg ctcacgatcc cagccggctt gcgctgcctg caacgattca 27300

ttggcaaggg cctgggcggc ttgccagagg gcggccacgg cctggttgcc ggcctgctgc 27360

accgcgtccg gcacctggac tgccagcggg gcggcctgcg ccgtgcgctg gcgtcgccat 27420

tcgcgcatgc cggcgctggc gtcgttcatg ttgacgcggg cggccttacg cactgcatcc 27480

acggtcggga agttctcccg gtcgccttgc tcgaacagct cgtccgcagc cgcaaaaatg 27540

cggtcgcgcg tctctttgtt cagttccatg ttggctccgg taattggtaa gaataataat 27600

actcttacct accttatcag cgcaagagtt tagctgaaca gttctcgact taacggcagg 27660

ttttttagcg gctgaagggc aggcaaaaaa agccccgcac ggtcggcggg ggcaaagggt 27720

cagcgggaag gggattagcg ggcgtcgggc ttcttcatgc gtcggggccg cgcttcttgg 27780

gatggagcac gacgaagcgc gcacgcgcat cgtcctcggc cctatcggcc cgcgtcgcgg 27840

tcaggaactt gtcgcgcgct aggtcctccc tggtgggcac caggggcatg aactcggcct 27900

gctcgatgta ggtccactcc atgaccgcat cgcagtcgag gccgcgttcc ttcaccgtct 27960

cttgcaggtc gcggtacgcc cgctcgttga gcggctggta acgggccaat tggtcgtaaa 28020

tggctgtcgg ccatgagcgg cctttcctgt tgagccagca gccgacgacg aagccggcaa 28080

tgcaggcccc tggcacaacc aggccgacgc cgggggcagg ggatggcagc agctcgccaa 28140

ccaggaaccc cgccgcgatg atgccgatgc cggtcaacca gcccttgaaa ctatccggcc 28200

ccgaaacacc cctgcgcatt gcctggatgc tgcgccggat agcttgcaac atcaggagcc 28260

gtttcttttg ttcgtcagtc atggtccgcc ctcaccagtt gttcgtatcg gtgtcggacg 28320

aactgaaatc gcaagagctg ccggtatcgg tccagccgct gtccgtgtcg ctgctgccga 28380

agcacggcga ggggtccgcg aacgccgcag acggcgtatc cggccgcagc gcatcgccca 28440

gcatggcccc ggtcagcgag ccgccggcca ggtagcccag catggtgctg ttggtcgccc 28500

cggccaccag ggccgacgtg acgaaatcgc cgtcattccc tctggattgt tcgctgctcg 28560

gcggggcagt gcgccgcgcc ggcggcgtcg tggatggctc gggttggctg gcctgcgacg 28620

gccggcgaaa ggtgcgcagc agctcgttat cgaccggctg cggcgtcggg gccgccgcct 28680

tgcgctgcgg tcggtgttcc ttcttcggct cgcgcagctt gaacagcatg atcgcggaaa 28740

ccagcagcaa cgccgcgcct acgcctcccg cgatgtagaa cagcatcgga ttcattcttc 28800

ggtcctcctt gtagcggaac cgttgtctgt gcggcgcggg tggcccgcgc cgctgtcttt 28860

ggggatcagc cctcgatgag cgcgaccagt ttcacgtcgg caaggttcgc ctcgaactcc 28920

tggccgtcgt cctcgtactt caaccaggca tagccttccg ccggcggccg acggttgagg 28980

-continued

ataaggcggg cagggcgctc gtcgtgctcg acctggacga tggccttttt cagcttgtcc 29040

gggtccggct ccttcgcgcc cttttccttg gcgtccttac cgtcctggtc gccgtcctcg 29100

ccgtcctggc cgtcgccggc ctccgcgtca cgctcggcat cagtctggcc gttgaaggca 29160

tcgacggtgt tgggatcgcg gcccttctcg tccaggaact cgcgcagcag cttgaccgtg 29220

ccgcgcgtga tttcctgggt gtcgtcgtca agccacgcct cgacttcctc cgggcgcttc 29280

ttgaaggccg tcaccagctc gttcaccacg gtcacgtcgc gcacgcggcc ggtgttgaac 29340

gcatcggcga tcttctccgg caggtccagc agcgtgacgt gctgggtgat gaacgccggc 29400

gacttgccga tttccttggc gatatcgcct ttcttcttgc ccttcgccag ctcgcggcca 29460

atgaagtcgg caatttcgcg cggggtcagc tcgttgcgtt gcaggttctc gataacctgg 29520

tcggcttcgt tgtagtcgtt gtcgatgaac gccgggatgg acttcttgcc ggcccacttc 29580

gagccacggt agcggcgggc gccgtgattg atgatatagc ggcccggctg ctcctggttc 29640

tcgcgcaccg aaatgggtga cttcaccccg cgctctttga tcgtggcacc gatttccgcg 29700

atgctctccg gggaaaagcc ggggttgtcg gccgtccgcg gctgatgcgg atcttcgtcg 29760

atcaggtcca ggtccagctc gatagggccg gaaccgccct gagacgccgc aggagcgtcc 29820

aggaggctcg acaggtcgcc gatgctatcc aaccccaggc cggacggctg cgccgcgcct 29880

gcggcttcct gagcggccgc agcggtgttt ttcttggtgg tcttggcttg agccgcagtc 29940

attgggaaat ctccatcttc gtgaacacgt aatcagccag ggcgcgaacc tctttcgatg 30000

ccttgcgcgc ggccgttttc ttgatcttcc agaccggcac accggatgcg agggcatcgg 30060

cgatgctgct gcgcaggcca acggtggccg gaatcatcat cttggggtac gcggccagca 30120

gctcggcttg gtggcgcgcg tggcgcggat tccgcgcatc gaccttgctg ggcaccatgc 30180

caaggaattg cagcttggcg ttcttctggc gcacgttcgc aatggtcgtg accatcttct 30240

tgatgccctg gatgctgtac gcctcaagct cgatggggga cagcacatag tcggccgcga 30300

agagggcggc cgccaggccg acgccaaggg tcggggccgt gtcgatcagg cacacgtcga 30360

agccttggtt cgccagggcc ttgatgttcg ccccgaacag ctcgcgggcg tcgtccagcg 30420

acagccgttc ggcgttcgcc agtaccgggt tggactcgat gagggcgagg cgcgcggcct 30480

ggccgtcgcc ggctgcgggt gcggtttcgg tccagccgcc ggcagggaca gcgccgaaca 30540

gcttgcttgc atgcaggccg gtagcaaagt ccttgagcgt gtaggacgca ttgccctggg 30600

ggtccaggtc gatcacggca acccgcaagc cgcgctcgaa aaagtcgaag gcaagatgca 30660

caagggtcga agtcttgccg acgccgcctt tctggttggc cgtgaccaaa gttttcatcg 30720

tttggtttcc tgtttttttct tggcgtccgc ttcccacttc cggacgatgt acgcctgatg 30780

ttccggcaga accgccgtta cccgcgcgta cccctcgggc aagttcttgt cctcgaacgc 30840

ggcccacacg cgatgcaccg cttgcgacac tgcgcccctg gtcagtccca gcgacgttgc 30900

gaacgtcgcc tgtggcttcc catcgactaa gacgccccgc gctatctcga tggtctgctg 30960

ccccacttcc agcccctgga tcgcctcctg gaactggctt tcggtaagcc gtttcttcat 31020

ggataacacc cataatttgc tccgcgcctt ggttgaacat agcggtgaca gccgccagca 31080

catgagagaa gtttagctaa acatttctcg cacgtcaaca cctttagccg ctaaaactcg 31140

tccttggcgt aacaaaacaa aagcccggaa accgggcttt cgtctcttgc cgcttatggc 31200

tctgcacccg gctccatcac caacaggtcg cgcacgcgct tcactcggtt gcggatcgac 31260

-continued

actgccagcc caacaaagcc ggttgccgcc gccgccagga tcgcgccgat gatgccggcc 31320

acaccggcca tcgcccacca ggtcgccgcc ttccggttcc attcctgctg gtactgcttc 31380

gcaatgctgg acctcggctc accataggct gaccgctcga tggcgtatgc cgcttctccc 31440

cttggcgtaa aacccagcgc cgcaggcggc attgccatgc tgcccgccgc tttcccgacc 31500

acgacgcgcg caccaggctt gcggtccaga ccttcggcca cggcgagctg cgcaaggaca 31560

taatcagccg ccgacttggc tccacgcgcc tcgatcagct cttgcactcg cgcgaaatcc 31620

ttggcctcca cggccgccat gaatcgcgca cgcggcgaag gctccgcagg gccggcgtcg 31680

tgatcgccgc cgagaatgcc cttcaccaag ttcgacgaca cgaaaatcat gctgacggct 31740

atcaccatca tgcagacgga tcgcacgaac ccgctgaatt gaacacgagc acggcacccg 31800

cgaccactat gccaagaatg cccaaggtaa aaattgccgg ccccgccatg aagtccgtga 31860

atgccccgac ggccgaagtg aagggcaggc cgccacccag gccgccgccc tcactgcccg 31920

gcacctggtc gctgaatgtc gatgccagca cctgcggcac gtcaatgctt ccgggcgtcg 31980

cgctcgggct gatcgcccat cccgttactg ccccgatccc ggcaatggca aggactgcca 32040

gcgctgccat ttttggggtg aggccgttcg cggccgaggg gcgcagcccc tgggggggatg 32100

ggaggcccgc gttagcgggc cgggagggtt cgagaagggg gggcaccccc cttcggcgtg 32160

cgcggtcacg cgcacagggc gcagccctgg ttaaaaacaa ggtttataaa tattggttta 32220

aaagcaggtt aaaagacagg ttagcggtgg ccgaaaaacg ggcggaaacc cttgcaaatg 32280

ctggattttc tgcctgtgga cagcccctca aatgtcaata ggtgcgcccc tcatctgtca 32340

gcactctgcc cctcaagtgt caaggatcgc gcccctcatc tgtcagtagt cgcgcccctc 32400

aagtgtcaat accgcagggc acttatcccc aggcttgtcc acatcatctg tgggaaactc 32460

gcgtaaaatc aggcgttttc gccgatttgc gaggctggcc agctccacgt cgccggccga 32520

aatcgagcct gcccctcatc tgtcaacgcc gcgccgggtg agtcggcccc tcaagtgtca 32580

acgtccgccc ctcatctgtc agtgagggcc aagttttccg cgaggtatcc acaacgccgg 32640

cggccgcggt gtctcgcaca cggcttcgac ggcgtttctg gcgcgtttgc agggccatag 32700

acggccgcca gcccagcggc gagggcaacc agcccggtga gcgtcggaaa ggcgctggaa 32760

gccccgtagc gacgcggaga ggggcgagac aagccaaggg cgcaggctcg atgcgcagca 32820

cgacatagcc ggttctcgca aggacgagaa tttccctgcg gtgcccctca agtgtcaatg 32880

aaagtttcca acgcgagcca ttcgcgagag ccttgagtcc acgctagatg agagctttgt 32940

tgtaggtgga ccagttggtg attttgaact tttgctttgc cacggaacgg tctgcgttgt 33000

cgggaagatg cgtgatctga tccttcaact cagcaaaagt tcgatttatt caacaaagcc 33060

acgttgtgtc tcaaaatctc tgatgttaca ttgcacaaga taaaaatata tcatcatgaa 33120

caataaaact gtctgcttac ataaacagta atacaagggg tgttatgagc catattcaac 33180

gggaaacgtc ttgctcgact ctagagctcg ttcctcgagg cctcgaggcc tcgaggaacg 33240

gtacctgcgg ggaagcttac aataatgtgt gttgttaagt cttgttgcct gtcatcgtct 33300

gactgacttt cgtcataaat cccggcctcc gtaacccagc tttgggcaag ctcacggatt 33360

tgatccggcg gaacgggaat atcgagatgc cgggctgaac gctgcagttc cagctttccc 33420

tttcgggaca ggtactccag ctgattgatt atctgctgaa gggtcttggt tccacctcct 33480

ggcacaatgc gaatgattac ttgagcgcga tcgggcatcc aattttctcc cgtcaggtgc 33540

-continued

gtggtcaagt gctacaaggc acctttcagt aacgagcgac cgtcgatccg tcgccgggat 33600

acggacaaaa tggagcgcag tagtccatcg agggcggcga aagcctcgcc aaaagcaata 33660

cgttcatctc gcacagcctc cagatccgat cgagggtctt cggcgtaggc agatagaagc 33720

atggatacat tgcttgagag tattccgatg gactgaagta tggcttccat cttttctcgt 33780

gtgtctgcat ctatttcgag aaagcccccg atgcggcgca ccgcaacgcg aattgccata 33840

ctatccgaaa gtcccagcag gcgcgcttga taggaaaagg tttcatactc ggccgatcgc 33900

agacgggcac tcacgacctt gaacccttca actttcaggg atcgatgctg gttgatggta 33960

gtctcactcg acgtggctct ggtgtgtttt gacatagctt cctccaaaga aagcggaagg 34020

tctggatact ccagcacgaa atgtgcccgg gtagacggat ggaagtctag ccctgctcaa 34080

tatgaaatca acagtacatt tacagtcaat actgaatata cttgctacat ttgcaattgt 34140

cttataacga atgtgaaata aaaatagtgt aacaacgctt ttactcatcg ataatcacaa 34200

aaacatttat acgaacaaaa atacaaatgc actccggttt cacaggatag gcgggatcag 34260

aatatgcaac ttttgacgtt ttgttctttc aaaggggggtg ctggcaaaac caccgcactc 34320

atgggccttt gcgctgcttt ggcaaatgac ggtaaacgag tggccctctt tgatgccgac 34380

gaaaaccggc ctctgacgcg atggagagaa aacgccttac aaagcagtac tgggatcctc 34440

gctgtgaagt ctattccgcc gacgaaatgc cccttcttga agcagcctat gaaaatgccg 34500

agctcgaagg atttgattat gcgttggccg atacgcgtgg cggctcgagc gagctcaaca 34560

acacaatcat cgctagctca aacctgcttc tgatccccac catgctaacg ccgctcgaca 34620

tcgatgaggc actatctacc taccgctacg tcatcgagct gctgttgagt gaaaatttgg 34680

caattcctac agctgttttg cgccaacgcg tcccggtcgg ccgattgaca acatcgcaac 34740

gcaggatgtc agagacgcta gagagccttc cagttgtacc gtctcccatg catgaaagag 34800

atgcatttgc cgcgatgaaa gaacgcggca tgttgcatct tacattacta aacacgggaa 34860

ctgatccgac gatgcgcctc atagagagga atcttcggat tgcgatggag gaagtcgtgg 34920

tcatttcgaa actgatcagc aaaatcttgg aggcttgaag atggcaattc gcaagcccgc 34980

attgtcggtc ggcgaagcac ggcggcttgc tggtgctcga cccgagatcc accatcccaa 35040

cccgacactt gttccccaga agctggacct ccagcacttg cctgaaaaag ccgacgagaa 35100

agaccagcaa cgtgagcctc tcgtcgccga tcacatttac agtcccgatc gacaacttaa 35160

gctaactgtg gatgccctta gtccacctcc gtccccgaaa aagctccagg tttttctttc 35220

agcgcgaccg cccgcgcctc aagtgtcgaa aacatatgac aacctcgttc ggcaatacag 35280

tccctcgaag tcgctacaaa tgattttaag gcgcgcgttg gacgatttcg aaagcatgct 35340

ggcagatgga tcatttcgcg tggccccgaa aagttatccg atcccttcaa ctacagaaaa 35400

atccgttctc gttcagacct cacgcatgtt cccggttgcg ttgctcgagg tcgctcgaag 35460

tcattttgat ccgttggggt tggagaccgc tcgagctttc ggccacaagc tggctaccgc 35520

cgcgctcgcg tcattctttg ctggagagaa gccatcgagc aattggtgaa gagggaccta 35580

tcggaacccc tcaccaaata ttgagtgtag gtttgaggcc gctggccgcg tcctcagtca 35640

ccttttgagc cagataatta agagccaaat gcaattggct caggctgcca tcgtcccccc 35700

gtgcgaaacc tgcacgtccg cgtcaaagaa ataaccggca cctcttgctg tttttatcag 35760

ttgagggctt gacggatccg cctcaagttt gcggcgcagc cgcaaaatga gaacatctat 35820

-continued

actcctgtcg taaacctcct cgtcgcgtac tcgactggca atgagaagtt gctcgcgcga 35880

tagaacgtcg cggggtttct ctaaaaacgc gaggagaaga ttgaactcac ctgccgtaag 35940

tttcacctca ccgccagctt cggacatcaa gcgacgttgc ctgagattaa gtgtccagtc 36000

agtaaaacaa aaagaccgtc ggtctttgga gcggacaacg ttggggcgca cgcgcaaggc 36060

aacccgaatg cgtgcaagaa actctctcgt actaaacggc ttagcgataa aatcacttgc 36120

tcctagctcg agtgcaacaa ctttatccgt ctcctcaagg cggtcgccac tgataattat 36180

gattggaata tcagactttg ccgccagatt tcgaacgatc tcaagcccat cttcacgacc 36240

taaatttaga tcaacaacca cgacatcgac cgtcgcggaa gagagtactc tagtgaactg 36300

ggtgctgtcg gctaccgcgg tcactttgaa ggcgtggatc gtaaggtatt cgataataag 36360

atgccgcata gcgacatcgt catcgataag aagaacgtgt ttcaacggct cacctttcaa 36420

tctaaaatct gaacccttgt tcacagcgct tgagaaattt tcacgtgaag gatgtacaat 36480

catctccagc taaatgggca gttcgtcaga attgcggctg accgcggatg acgaaaatgc 36540

gaaccaagta tttcaatttt atgacaaaag ttctcaatcg ttgttacaag tgaaacgctt 36600

cgaggttaca gctactattg attaaggaga tcgcctatgg tctcgccccg gcgtcgtgcg 36660

tccgccgcga gccagatctc gcctacttca taaacgtcct cataggcacg gaatggaatg 36720

atgacatcga tcgccgtaga gagcatgtca atcagtgtgc gatcttccaa gctagcacct 36780

tgggcgctac ttttgacaag ggaaaacagt ttcttgaatc cttggattgg attcgcgccg 36840

tgtattgttg aaatcgatcc cggatgtccc gagacgactt cactcagata agcccatgct 36900

gcatcgtcgc gcatctcgcc aagcaatatc cggtccggcc gcatacgcag acttgcttgg 36960

agcaagtgct cggcgctcac agcacccagc ccagcaccgt tcttggagta gagtagtcta 37020

acatgattat cgtgtggaat gacgagttcg agcgtatctt ctatggtgat tagcctttcc 37080

tgggggggga tggcgctgat caaggtcttg ctcattgttg tcttgccgct tccggtaggg 37140

ccacatagca acatcgtcag tcggctgacg acgcatgcgt gcagaaacgc ttccaaatcc 37200

ccgttgtcaa aatgctgaag gatagcttca tcatcctgat tttggcgttt ccttcgtgtc 37260

tgccactggt tccacctcga agcatcataa cgggaggaga cttctttaag accagaaaca 37320

cgcgagcttg gccgtcgaat ggtcaagctg acggtgcccg agggaacggt cggcggcaga 37380

cagatttgta gtcgttcacc accaggaagt tcagtggcgc agagggggtt acgtggtccg 37440

acatcctgct ttctcagcgc gcccgctaaa atagcgatat cttcaagatc atcataagag 37500

acgggcaaag gcatcttggt aaaaatgccg gcttggcgca caaatgcctc tccaggtcga 37560

ttgatcgcaa tttcttcagt cttcgggtca tcgagccatt ccaaaatcgg cttcagaaga 37620

aagcgtagtt gcggatccac ttccatttac aatgtatcct atctctaagc ggaaatttga 37680

attcattaag agcggcggtt cctcccccgc gtggcgccgc cagtcaggcg gagctggtaa 37740

acaccaaaga aatcgaggtc ccgtgctacg aaaatggaaa cggtgtcacc ctgattcttc 37800

ttcagggttg gcggtatgtt gatggttgcc ttaagggctg tctcagttgt ctgctcaccg 37860

ttattttgaa agctgttgaa gctcatcccg ccacccgagc tgccggcgta ggtgctagct 37920

gcctggaagg cgccttgaac aacactcaag agcatagctc cgctaaaacg ctgccagaag 37980

tggctgtcga ccgagcccgg caatcctgag cgaccgagtt cgtccgcgct tggcgatgtt 38040

aacgagatca tcgcatggtc aggtgtctcg gcgcgatccc acaacacaaa aacgcgccca 38100

-continued

```
tctccctgtt gcaagccacg ctgtatttcg ccaacaacgg tggtgccacg atcaagaagc  38160

acgatattgt tcgttgttcc acgaatatcc tgaggcaaga cacactttac atagcctgcc  38220

aaatttgtgt cgattgcggt ttgcaagatg cacggaatta ttgtcccttg cgttaccata  38280

aaatcggggt gcggcaagag cgtggcgctg ctgggctgca gctcggtggg tttcatacgt  38340

atcgacaaat cgttctcgcc ggacacttcg ccattcggca aggagttgtc gtcacgcttg  38400

ccttcttgtc ttcggcccgt gtcgccctga atggcgcgtt tgctgacccc ttgatcgccg  38460

ctgctatatg caaaaatcgg tgtttcttcc ggccgtggct catgccgctc cggttcgccc  38520

ctcggcggta gaggagcagc aggctgaaca gcctcttgaa ccgctggagg atccggcggc  38580

acctcaatcg gagctggatg aaatggcttg gtgtttgttg cgatcaaagt tgacggcgat  38640

gcgttctcat tcaccttctt ttggcgccca cctagccaaa tgaggcttaa tgataacgcg  38700

agaacgacac ctccgacgat caatttctga gaccccgaaa gacgccggcg atgtttgtcg  38760

gagaccaggg atccagatgc atcaacctca tgtgccgctt gctgactatc gttattcatc  38820

ccttcgcccc cttcaggacg cgtttcacat cgggcctcac cgtgcccgtt tgcggccttt  38880

ggccaacggg atcgtaagcg gtgttccaga tacatagtac tgtgtggcca tccctcagac  38940

gccaacctcg ggaaaccgaa gaaatctcga catcgctccc tttaactgaa tagttggcaa  39000

cagcttcctt gccatcagga ttgatggtgt agatggaggg tatgcgtaca ttgcccggaa  39060

agtggaatac cgtcgtaaat ccattgtcga agacttcgag tggcaacagc gaacgatcgc  39120

cttgggcgac gtagtgccaa ttactgtccg ccgcaccaag ggctgtgaca ggctgatcca  39180

ataaattctc agctttccgt tgatattgtg cttccgcgtg tagtctgtcc acaacagcct  39240

tctgttgtgc ctcccttcgc cgagccgccg catcgtcggc ggggtaggcg aattggacgc  39300

tgtaatagag atcgggctgc tctttatcga ggtgggacag agtcttggaa cttatactga  39360

aaacataacg gcgcatcccg gagtcgcttg cggttagcac gattactggc tgaggcgtga  39420

ggacctggct tgccttgaaa aatagataat ttccccgcgg tagggctgct agatctttgc  39480

tatttgaaac ggcaaccgct gtcaccgttt cgttcgtggc gaatgttacg accaaagtag  39540

ctccaaccgc cgtcgagagg cgcaccactt gatcgggatt gtaagccaaa taacgcatgc  39600

gcggatctag cttgcccgcc attggagtgt cttcagcctc cgcaccagtc gcagcggcaa  39660

ataaacatgc taaaatgaaa agtgcttttc tgatcatggt tcgctgtggc ctacgtttga  39720

aacggtatct tccgatgtct gataggaggt gacaaccaga cctgccgggt tggttagtct  39780

caatctgccg ggcaagctgg tcacctttтc gtagcgaact gtcgcggtcc acgtactcac  39840

cacaggcatt ttgccgtcaa cgacgagggt ccttttatag cgaatttgct gcgtgcttgg  39900

agttacatca tttgaagcga tgtgctcgac ctccaccctg ccgcgtttgc caagaatgac  39960

ttgaggcgaa ctgggattgg gatagttgaa gaattgctgg taatcctggc gcactgttgg  40020

ggcactgaag ttcgatacca ggtcgtaggc gtactgagcg gtgtcggcat cataactctc  40080

gcgcaggcga acgtactccc acaatgaggc gttaacgacg gcctcctctt gagttgcagg  40140

caatcgcgag acagacacct cgctgtcaac ggtgccgtcc ggccgtatcc atagatatac  40200

gggcacaagc ctgctcaacg gcaccattgt ggctatagcg aacgcttgag caacatttcc  40260

caaaatcgcg atagctgcga cagctgcaat gagtttggag agacgtcgcg ccgatttcgc  40320

tcgcgcggtt tgaaaggctt ctacttcctt atagtgctcg gcaaggcttt cgcgcgccac  40380
```

-continued

tagcatggca tattcaggcc ccgtcatagc gtccacccga attgccgagc tgaagatctg 40440

acggagtagg ctgccatcgc cccacattca gcgggaagat cgggcctttg cagctcgcta 40500

atgtgtcgtt tgtctggcag ccgctcaaag cgacaactag gcacagcagg caatacttca 40560

tagaattctc cattgaggcg aatttttgcg cgacctagcc tcgctcaacc tgagcgaagc 40620

gacggtacaa gctgctggca gattgggttg cgccgctcca gtaactgcct ccaatgttgc 40680

cggcgatcgc cggcaaagcg acaatgagcg catcccctgt cagaaaaaac atatcgagtt 40740

cgtaaagacc aatgatcttg gccgcggtcg taccggcgaa ggtgattaca ccaagcataa 40800

gggtgagcgc agtcgcttcg gttaggatga cgatcgttgc cacgaggttt aagaggagaa 40860

gcaagagacc gtaggtgata agttgcccga tccacttagc tgcgatgtcc cgcgtgcgat 40920

caaaaatata tccgacgagg atcagaggcc cgatcgcgag aagcactttc gtgagaattc 40980

caacggcgtc gtaaactccg aaggcagacc agagcgtgcc gtaaaggacc cactgtgccc 41040

cttggaaagc aaggatgtcc tggtcgttca tcggaccgat ttcggatgcg attttctgaa 41100

aaacggcctg ggtcacggcg aacattgtat ccaactgtgc cggaacagtc tgcagaggca 41160

agccggttac actaaactgc tgaacaaagt ttgggaccgt cttttcgaag atggaaacca 41220

catagtcttg gtagttagcc tgcccaacaa ttagagcaac aacgatggtg accgtgatca 41280

cccgagtgat accgctacgg gtatcgactt cgccgcgtat gactaaaata ccctgaacaa 41340

taatccaaag agtgacacag gcgatcaatg gcgcactcac cgcctcctgg atagtctcaa 41400

gcatcgagtc caagcctgtc gtgaaggcta catcgaagat cgtatgaatg gccgtaaacg 41460

gcgccggaat cgtgaaattc atcgattgga cctgaacttg actggtttgt cgcataatgt 41520

tggataaaat gagctcgcat tcggcgagga tgcgggcgga tgaacaaatc gcccagcctt 41580

aggggagggc accaaagatg acagcggtct tttgatgctc cttgcgttga gcggccgcct 41640

cttccgcctc gtgaaggccg gcctgcgcgg tagtcatcgt taataggctt gtcgcctgta 41700

cattttgaat cattgcgtca tggatctgct tgagaagcaa accattggtc acggttgcct 41760

gcatgatatt gcgagatcgg gaaagctgag cagacgtatc agcattcgcc gtcaagcgtt 41820

tgtccatcgt ttccagattg tcagccgcaa tgccagcgct gtttgcggaa ccggtgatct 41880

gcgatcgcaa caggtccgct tcagcatcac tacccacgac tgcacgatct gtatcgctgg 41940

tgatcgcacg tgccgtggtc gacattggca ttcgcggcga aaacatttca ttgtctaggt 42000

ccttcgtcga aggatactga tttttctggt tgagcgaagt cagtagtcca gtaacgccgt 42060

aggccgacgt caacatcgta accatcgcta tagtctgagt gagattctcc gcagtcgcga 42120

gcgcagtcgc gagcgtctca gcctccgttg ccgggtcgct aacaacaaac tgcgcccgcg 42180

cgggctgaat atatagaaag ctgcaggtca aaactgttgc aataagttgc gtcgtcttca 42240

tcgtttccta ccttatcaat cttctgcctc gtggtgacgg gccatgaatt cgctgagcca 42300

gccagatgag ttgccttctt gtgcctcgcg tagtcgagtt gcaaagcgca ccgtgttggc 42360

acgccccgaa agcacggcga catattcacg catatcccgc agatcaaatt cgcagatgac 42420

gcttccactt tctcgtttaa gaagaaactt acggctgccg accgtcatgt cttcacggat 42480

cgcctgaaat tccttttcgg tacatttcag tccatcgaca taagccgatc gatctgcggt 42540

tggtgatgga tagaaaatct tcgtcataca ttgcgcaacc aagctggctc ctagcggcga 42600

ttccagaaca tgctctggtt gctgcgttgc cagtattagc atcccgttgt tttttcgaac 42660

-continued

```
ggtcaggagg aatttgtcga cgacagtcga aaatttaggg tttaacaaat aggcgcgaaa  42720
ctcatcgcag ctcatcacaa aacggcggcc gtcgatcatg gctccaatcc gatgcaggag  42780
atatgctgca gcgggagcgc atacttcctc gtattcgaga agatgcgtca tgtcgaagcc  42840
ggtaatcgac ggatctaact ttacttcgtc aacttcgccg tcaaatgccc agccaagcgc  42900
atggccccgg caccagcgtt ggagccgcgc tcctgcgcct tcggcgggcc catgcaacaa  42960
aaattcacgt aaccccgcga ttgaacgcat ttgtggatca aacgagagct gacgatggat  43020
accacggacc agacggcggt tctcttccgg agaaatccca ccccgaccat cactctcgat  43080
gagagccacg atccattcgc gcagaaaatc gtgtgaggct gctgtgtttt ctaggccacg  43140
caacggcgcc aacccgctgg gtgtgcctct gtgaagtgcc aaatatgttc ctcctgtggc  43200
gcgaaccagc aattcgccac cccggtcctt gtcaaagaac acgaccgtac ctgcacggtc  43260
gaccatgctc tgttcgagca tggctagaac aaacatcatg agcgtcgtct tacccctccc  43320
gataggcccg aatattgccg tcatgccaac atcgtgctca tgcgggatat agtcgaaagg  43380
cgttccgcca ttggtacgaa atcgggcaat cgcgttgccc cagtggcctg agctggcgcc  43440
ctctggaaag ttttcgaaag agacaaaccc tgcgaaattg cgtgaagtga ttgcgccagg  43500
gcgtgtgcgc cacttaaaat tccccggcaa ttgggaccaa taggccgctt ccataccaat  43560
accttcttgg acaaccacgg cacctgcatc cgccattcgt gtccgagccc gcgcgcccct  43620
gtccccaaga ctattgagat cgtctgcata gacgcaaagg ctcaaatgat gtgagcccat  43680
aacgaattcg ttgctcgcaa gtgcgtcctc agcctcggat aatttgccga tttgagtcac  43740
ggctttatcg ccggaactca gcatctggct cgatttgagg ctaagtttcg cgtgcgcttg  43800
cgggcgagtc aggaacgaaa aactctgcgt gagaacaagt ggaaaatcga gggatagcag  43860
cgcgttgagc atgcccggcc gtgtttttgc agggtattcg cgaaacgaat agatggatcc  43920
aacgtaactg tcttttggcg ttctgatctc gagtcctcgc ttgccgcaaa tgactctgtc  43980
ggtataaatc gaagcgccga gtgagccgct gacgaccgga accggtgtga accgaccagt  44040
catgatcaac cgtagcgctt cgccaatttc ggtgaagagc acaccctgct tctcgcggat  44100
gccaagacga tgcaggccat acgctttaag agagccagcg acaacatgcc aaagatcttc  44160
catgttcctg atctggcccg tgagatcgtt ttccctttt ccgcttagct tggtgaacct  44220
cctctttacc ttccctaaag ccgcctgtgg gtagacaatc aacgtaagga agtgttcatt  44280
gcggaggagt tggccggaga gcacgcgctg ttcaaaagct tcgttcaggc tagcggcgaa  44340
aacactacgg aagtgtcgcg gcgccgatga tggcacgtcg gcatgacgta cgaggtgagc  44400
atatattgac acatgatcat cagcgatatt gcgcaacagc gtgttgaacg cacgacaacg  44460
cgcattgcgc atttcagttt cctcaagctc gaatgcaacg ccatcaattc tcgcaatggt  44520
catgatcgat ccgtcttcaa gaaggacgat atggtcgctg aggtggccaa tataagggag  44580
atagatctca ccggatcttt cggtcgttcc actcgcgccg agcatcacac cattcctctc  44640
cctcgtgggg gaaccctaat tggatttggg ctaacagtag cgccccccca aactgcacta  44700
tcaatgcttc ttcccgcggt ccgcaaaaat agcaggacga cgctcgccgc attgtagtct  44760
cgctccacga tgagccgggc tgcaaaccat aacggcacga gaacgacttc gtagagcggg  44820
ttctgaacga taacgatgac aaagccggcg aacatcatga ataaccctgc caatgtcagt  44880
ggcaccccaa gaaacaatgc gggccgtgtg gctgcgaggt aaagggtcga ttcttccaaa  44940
```

-continued

cgatcagcca tcaactaccg ccagtgagcg tttggccgag gaagctcgcc ccaaacatga 45000

taacaatgcc gccgacgacg ccggcaacca gcccaagcga agcccgcccg aacatccagg 45060

agatcccgat agcgacaatg ccgagaacag cgagtgactg gccgaacgga ccaaggataa 45120

acgtgcatat attgttaacc attgtggcgg ggtcagtgcc gccacccgca gattgcgctg 45180

cggcgggtcc ggatgaggaa atgctccatg caattgcacc gcacaagctt ggggcgcagc 45240

tcgatatcac gcgcatcatc gcattcgaga gcgagaggcg atttagatgt aaacggtatc 45300

tctcaaagca tcgcatcaat gcgcacctcc ttagtataag tcgaataaga cttgattgtc 45360

gtctgcggat ttgccgttgt cctggtgtgg cggtggcgga gcgattaaac cgccagcgcc 45420

atcctcctgc gagcggcgct gatatgaccc ccaaacatcc cacgtctctt cggattttag 45480

cgcctcgtga tcgtcttttg gaggctcgat taacgcgggc accagcgatt gagcagctgt 45540

ttcaactttt cgcacgtagc cgtttgcaaa accgccgatg aaattaccgg tgttgtaagc 45600

ggagatcgcc cgacgaagcg caaattgctt ctcgtcaatc gtttcgccgc ctgcataacg 45660

acttttcagc atgtttgcag cggcagataa tgatgtgcac gcctggagcg caccgtcagg 45720

tgtcagaccg agcatagaaa aatttcgaga gtttatttgc atgaggccaa catccagcga 45780

atgccgtgca tcgagacggt gcctgacgac ttgggttgct tggctgtgat cttgccagtg 45840

aagcgtttcg ccggtcgtgt tgtcatgaat cgctaaagga tcaaagcgac tctccacctt 45900

agctatcgcc gcaagcgtag atgtcgcaac tgatggggca cacttgcgag caacatggtc 45960

aaactcagca gatgagagtg gcgtggcaag gctcgacgaa cagaaggaga ccatcaaggc 46020

aagagaaagc gaccccgatc tcttaagcat accttatctc cttagctcgc aactaacacc 46080

gcctctcccg ttggaagaag tgcgttgttt tatgttgaag attatcggga gggtcggtta 46140

ctcgaaaatt ttcaattgct tctttatgat ttcaattgaa gcgagaaacc tcgcccggcg 46200

tcttggaacg caacatggac cgagaaccgc gcatccatga ctaagcaacc ggatcgacct 46260

attcaggccg cagttggtca ggtcaggctc agaacgaaaa tgctcggcga ggttacgctg 46320

tctgtaaacc cattcgatga acgggaagct tccttccgat tgctcttggc aggaatattg 46380

gcccatgcct gcttgcgctt tgcaaatgct cttatcgcgt tggtatcata tgccttgtcc 46440

gccagcagaa acgcactcta agcgattatt tgtaaaaatg tttcggtcat gcggcggtca 46500

tgggcttgac ccgctgtcag cgcaagacgg atcggtcaac cgtcggcatc gacaacagcg 46560

tgaatcttgg tggtcaaacc gccacgggaa cgtcccatac agccatcgtc ttgatcccgc 46620

tgtttcccgt cgccgcatgt tggtggacgc ggacacagga actgtcaatc atgacgacat 46680

tctatcgaaa gccttggaaa tcacactcag aatatgatcc cagacgtctg cctcacgcca 46740

tcgtacaaag cgattgtagc aggttgtaca ggaaccgtat cgatcaggaa cgtctgccca 46800

gggcgggccc gtccggaagc gccacaagat gacattgatc acccgcgtca acgcgcggca 46860

cgcgacgcgg cttatttggg aacaaaggac tgaacaacag tccattcgaa atcggtgaca 46920

tcaaagcggg gacgggttat cagtggcctc caagtcaagc ctcaatgaat caaaatcaga 46980

ccgatttgca aacctgattt atgagtgtgc ggcctaaatg atgaaatcgt ccttctagat 47040

cgcctccgtg gtgtagcaac acctcgcagt atcgccgtgc tgaccttggc cagggaattg 47100

actggcaagg gtgctttcac atgaccgctc ttttggccgc gatagatgat ttcgttgctg 47160

ctttgggcac gtagaaggag agaagtcata tcggagaaat tcctcctggc gcgagagcct 47220

-continued

gctctatcgc gacggcatcc cactgtcggg aacagaccgg atcattcacg aggcgaaagt 47280

cgtcaacaca tgcgttatag gcatcttccc ttgaaggatg atcttgttgc tgccaatctg 47340

gaggtgcggc agccgcaggc agatgcgatc tcagcgcaac ttgcggcaaa acatctcact 47400

cacctgaaaa ccactagcga gtctcgcgat cagacgaagg ccttttactt aacgacacaa 47460

tatccgatgt ctgcatcaca ggcgtcgcta tcccagtcaa tactaaagcg gtgcaggaac 47520

taaagattac tgatgactta ggcgtgccac gaggcctgag acgacgcgcg tagacagttt 47580

tttgaaatca ttatcaaagt gatggcctcc gctgaagcct atcacctctg cgccggtctg 47640

tcggagagat gggcaagcat tattacggtc ttcgcgcccg tacatgcatt ggacgattgc 47700

agggtcaatg gatctgagat catccagagg attgccgccc ttaccttccg tttcgagttg 47760

gagccagccc ctaaatgaga cgacatagtc gacttgatgt gacaatgcca agagagagat 47820

ttgcttaacc cgatttttt gctcaagcgt aagcctattg aagcttgccg gcatgacgtc 47880

cgcgccgaaa gaatatccta caagtaaaac attctgcaca ccgaaatgct tggtgtagac 47940

atcgattatg tgaccaagat ccttagcagt ttcgcttggg gaccgctccg accagaaata 48000

ccgaagtgaa ctgacgccaa tgacaggaat cccttccgtc tgcagatagg taccatcgat 48060

agatctgctg cctcgcgcgt ttcggtgatg acggtgaaaa cctctgacac atgcagctcc 48120

cggagacggt cacagcttgt ctgtaagcgg atgccgggag cagacaagcc cgtcagggcg 48180

cgtcagcggg tgttggcggg tgtcggggcg cagccatgac ccagtcacgt agcgatagcg 48240

gagtgtatac tggcttaact atgcggcatc agagcagatt gtactgagag tgcaccatat 48300

gcggtgtgaa ataccgcaca gatgcgtaag gagaaaatac cgcatcaggc gctcttccgc 48360

ttcctcgctc actgactcgc tgcgctcggt cgttcggctg cggcgagcgg tatcagctca 48420

ctcaaaggcg gtaatacggt tatccacaga atcaggggat aacgcaggaa agaacatgtg 48480

agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca 48540

taggctccgc ccccctgacg agcatcacaa aaatcgacgc tcaagtcaga ggtggcgaaa 48600

cccgacagga ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc 48660

tgttccgacc ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc 48720

gctttctcat agctcacgct gtaggtatct cagttcggtg taggtcgttc gctccaagct 48780

gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg 48840

tcttgagtcc aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag 48900

gattagcaga gcgaggtatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta 48960

cggctacact agaaggacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg 49020

aaaaagagtt ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt 49080

tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt 49140

ttctacgggg tctgacgctc agtggaacga aaactcacgt taagggattt tggtcatgag 49200

attatcaaaa aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat 49260

ctaaagtata tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc 49320

tatctcagcg atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat 49380

aactacgata cgggagggct taccatctgg ccccagtgct gcaatgatac cgcgagaccc 49440

acgctcaccg gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgcag 49500

-continued

aagtggtcct gcaactttat ccgcctccat ccagtctatt aattgttgcc gggaagctag 49560

agtaagtagt tcgccagtta atagtttgcg caacgttgtt gccattgctg caggggggg 49620

ggggggggg ttccattgtt cattccacgg acaaaaacag agaaaggaaa cgacagaggc 49680

caaaaagctc gctttcagca cctgtcgttt cctttctttt cagagggtat tttaaataaa 49740

aacattaagt tatgacgaag aagaacggaa acgccttaaa ccggaaaatt ttcataaata 49800

gcgaaaaccc gcgaggtcgc cgccccgtac tgtcggatca ccggaaagga cccgtaaagt 49860

gataatgatt atcatctaca tatcacaacg tgcgtggagg ccatcaaacc acgtcaaata 49920

atcaattatg acgcaggtat cgtattaatt gatctgcatc aacttaacgt aaaaacaact 49980

tcagacaata caaatcagcg acactgaata cggggcaacc tcatgtcccc ccccccccc 50040

cccctgcagg catcgtggtg tcacgctcgt cgtttggtat ggcttcattc agctccggtt 50100

cccaacgatc aaggcgagtt acatgatccc ccatgttgtg caaaaaagcg gttagctcct 50160

tcggtcctcc gatcgttgtc agaagtaagt tggccgcagt gttatcactc atggttatgg 50220

cagcactgca taattctctt actgtcatgc catccgtaag atgcttttct gtgactggtg 50280

agtactcaac caagtcattc tgagaatagt gtatgcggcg accgagttgc tcttgcccgg 50340

cgtcaacacg ggataatacc gcgccacata gcagaacttt aaaagtgctc atcattggaa 50400

aacgttcttc ggggcgaaaa ctctcaagga tcttaccgct gttgagatcc agttcgatgt 50460

aacccactcg tgcacccaac tgatcttcag catcttttac tttcaccagc gtttctgggt 50520

gagcaaaaac aggaaggcaa aatgccgcaa aaaagggaat aagggcgaca cggaaatgtt 50580

gaatactcat actcttcctt tttcaatatt attgaagcat ttatcagggt tattgtctca 50640

tgagcggata catatttgaa tgtatttaga aaaataaaca aatagggtt ccgcgcacat 50700

ttccccgaaa agtgccacct gacgtctaag aaaccattat tatcatgaca ttaacctata 50760

aaaataggcg tatcacgagg ccctttcgtc ttcaagaatt ggtcgacgat cttgctgcgt 50820

tcggatattt tcgtggagtt cccgccacag acccggattg aaggcgagat ccagcaactc 50880

gcgccagatc atcctgtgac ggaactttgg cgcgtgatga ctggccagga cgtcggccga 50940

aagagcgaca agcagatcac gcttttcgac agcgtcggat ttgcgatcga ggatttttcg 51000

gcgctgcgct acgtccgcga ccgcgttgag ggatcaagcc acagcagccc actcgacctt 51060

ctagccgacc cagacgagcc aagggatctt tttggaatgc tgctccgtcg tcaggctttc 51120

cgacgtttgg gtggttgaac agaagtcatt atcgtacgga atgccaagca ctcccgaggg 51180

gaaccctgtg gttggcatgc acatacaaat ggacgaacgg ataaaccttt tcacgccctt 51240

ttaaatatcc gttattctaa taaacgctct tttctcttag 51280

<210> SEQ ID NO 4
<211> LENGTH: 1603
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: see Table 1 of specification

<400> SEQUENCE: 4

tagcagacgc ggaaccagcc gggctcccgg cagtggcagg aggagcccgg ggagatgttg 60

agccccacct cgaagaccac cctcttccac agctccatct cgccctcgaa cgaccggctc 120

cgcatcaggc gccgcatgtt gacccagcag aagagccccg cgttgctctc caggcactcg 180

-continued

atgcccacgg ccgccaggcc ctccgccagc tgctcgcgcc gctccctgat ccgccgcgtg 240

ttctccgcga tgtacctccg cgtgaagtcc ctgtcgccca ggagcgacgc caggaggtgc 300

tgcgtctggg acgacaccag gccgaagctc gacatcttgg tggccgcgga gaccacgccg 360

gcgttggacg agtagatggc gcccacgcgg aaccccggga ggcccaggtc cttggacagg 420

ctgtacacca cgtgcacgcg gtccgacagc ggcccaacgc cgacgacgcc gtcgtccgtg 480

gcggcgcgcg cggccaccac ctgcagtcga cgtgcaaagg tccgccttgt ttctcctctg 540

tctcttgatc tgactaatct tggtttatga ttcgttgagt aattttgggg aaagcttcgt 600

ccacagtttt ttttcgatga acagtgccgc agtggcgctg atcttgtatg ctatcctgca 660

atcgtggtga acttatttct tttatatcct ttactcccat gaaaaggcta gtaatctttc 720

tcgatgtaac atcgtccagc actgctatta ccgtgtggtc catccgacag tctggctgaa 780

cacatcatac gatctatgga gcaaaaatct atcttccctg ttctttaatg aaggacgtca 840

ttttcattag tatgatctag gaatgttgca acttgcaagg aggcgtttct ttctttgaat 900

ttaactaact cgttgagtgg ccctgtttct cggacgtaag gcctttgctg ctccacacat 960

gtccattcga attttaccgt gtttagcaag ggcgaaaagt ttgcatcttg atgatttagc 1020

ttgactatgc gattgctttc ctggacccgt gcagctggat cccggtacgc gccgccacgg 1080

acgacggcgt cgtcggcgtt gggccgctgt cggaccgcgt gcacgtggtg tacagcctgt 1140

ccaaggacct gggcctcccg gggttccgcg tgggcgccat ctactcgtcc aacgccggcg 1200

tggtctccgc ggccaccaag atgtcgagct tcggcctggt gtcgtcccag acgcagcacc 1260

tcctggcgtc gctcctgggc gacagggact tcacgcggag gtacatcgcg gagaacacgc 1320

ggcggatcag ggagcggcgc gagcagctgg cggagggcct ggcggccgtg ggcatcgagt 1380

gcctggagag caacgcgggg ctcttctgct gggtcaacat gcggcgcctg atgcggagcc 1440

ggtcgttcga gggcgagatg gagctgtgga agagggtggt cttcgaggtg gggctcaaca 1500

tctccccggg ctcctcctgc cactgccggg agcccggctg gttccgcgtc tgctaaaggg 1560

cgaattccag cacactggcg gccgttacta gtggatccga gct 1603

<210> SEQ ID NO 5
<211> LENGTH: 3657
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: see Table 1 of specification

<400> SEQUENCE: 5

gtgcagcgtg acccggtcgt gcccctctct agagataatg agcattgcat gtctaagtta 60

taaaaaatta ccacatattt tttttgtcac acttgtttga agtgcagttt atctatcttt 120

atacatatat ttaaacttta ctctacgaat aatataatct atagtactac aataatatca 180

gtgttttaga gaatcatata aatgaacagt tagacatggt ctaaaggaca attgagtatt 240

ttgacaacag gactctacag ttttatcttt ttagtgtgca tgtgttctcc tttttttttg 300

caaatagctt cacctatata atacttcatc cattttatta gtacatccat ttagggttta 360

gggttaatgg tttttataga ctaatttttt tagtacatct attttattct attttagcct 420

ctaaattaag aaaactaaaa ctctatttta gtttttttat ttaataattt agatataaaa 480

tagaataaaa taaagtgact aaaaattaaa caaataccct ttaagaaatt aaaaaaacta 540

-continued

aggaaacatt tttcttgttt cgagtagata atgccagcct gttaaacgcc gtcgacgagt 600

ctaacggaca ccaaccagcg aaccagcagc gtcgcgtcgg gccaagcgaa gcagacggca 660

cggcatctct gtcgctgcct ctggacccct ctcgagagtt ccgctccacc gttggacttg 720

ctccgctgtc ggcatccaga aattgcgtgg cggagcggca gacgtgagcc ggcacggcag 780

gcggcctcct cctcctctca cggcaccggc agctacgggg gattcctttc ccaccgctcc 840

ttcgctttcc cttcctcgcc cgccgtaata aatagacacc ccctccacac cctctttccc 900

caacctcgtg ttgttcggag cgcacacaca cacaaccaga tctcccccaa atccacccgt 960

cggcacctcc gcttcaaggt acgccgctcg tcctcccccc ccccctctc taccttctct 1020

agatcggcgt tccggtccat gcatggttag ggcccggtag ttctacttct gttcatgttt 1080

gtgttagatc cgtgtttgtg ttagatccgt gctgctagcg ttcgtacacg gatgcgacct 1140

gtacgtcaga cacgttctga ttgctaactt gccagtgttt ctctttgggg aatcctggga 1200

tggctctagc cgttccgcag acgggatcga tttcatgatt ttttttgttt cgttgcatag 1260

ggtttggttt gccctttcc tttatttcaa tatatgccgt gcacttgttt gtcgggtcat 1320

cttttcatgc tttttttgt cttggttgtg atgatgtggt ctggttgggc ggtcgttcta 1380

gatcggagta gaattctgtt tcaaactacc tggtggattt attaattttg gatctgtatg 1440

tgtgtgccat acatattcat agttacgaat tgaagatgat ggatggaaat atcgatctag 1500

gataggtata catgttgatg cgggttttac tgatgcatat acagagatgc tttttgttcg 1560

cttggttgtg atgatgtggt gtggttgggc ggtcgttcat tcgttctaga tcggagtaga 1620

atactgtttc aaactacctg gtgtatttat taattttgga actgtatgtg tgtgtcatac 1680

atcttcatag ttacgagttt aagatggatg gaaatatcga tctaggatag gtatacatgt 1740

tgatgtgggt tttactgatg catatacatg atggcatatg cagcatctat tcatatgctc 1800

taaccttgag tacctatcta ttataataaa caagtatgtt ttataattat tttgatcttg 1860

atatacttgg atgatggcat atgcagcagc tatatgtgga ttttttttagc cctgccttca 1920

tacgctattt atttgcttgg tactgtttct tttgtcgatg ctcaccctgt tgtttggtgt 1980

tacttctgca ggtcgacttt aacttagcct aggatccact agtaacggcc gccagtgtgc 2040

tggaattcgc cctttagcag acgcggaacc agccgggctc ccggcagtgg caggaggagc 2100

ccggggagat gttgagcccc acctcgaaga ccaccctctt ccacagctcc atctcgccct 2160

cgaacgaccg gctccgcatc aggcgccgca tgttgaccca gcagaagagc cccgcgttgc 2220

tctccaggca ctcgatgccc acggccgcca ggccctccgc cagctgctcg cgccgctccc 2280

tgatccgccg cgtgttctcc gcgatgtacc tccgcgtgaa gtccctgtcg cccaggagcg 2340

acgccaggag gtgctgcgtc tgggacgaca ccaggccgaa gctcgacatc ttggtggccg 2400

cggagaccac gccggcgttg gacgagtaga tggcgcccac gcggaacccc gggaggccca 2460

ggtccttgga caggctgtac accacgtgca cgcggtccga cagcggccca acgccgacga 2520

cgccgtcgtc cgtggcggcg cgcgcggcca ccacctgcag tcgacgtgca aaggtccgcc 2580

ttgtttctcc tctgtctctt gatctgacta atcttggttt atgattcgtt gagtaatttt 2640

ggggaaagct tcgtccacag tttttttttcg atgaacagtg ccgcagtggc gctgatcttg 2700

tatgctatcc tgcaatcgtg gtgaacttat ttcttttata tcctttactc ccatgaaaag 2760

gctagtaatc tttctcgatg taacatcgtc cagcactgct attaccgtgt ggtccatccg 2820

-continued

acagtctggc tgaacacatc atacgatcta tggagcaaaa atctatcttc cctgttcttt 2880

aatgaaggac gtcattttca ttagtatgat ctaggaatgt tgcaacttgc aaggaggcgt 2940

ttctttcttt gaatttaact aactcgttga gtggccctgt ttctcggacg taaggccttt 3000

gctgctccac acatgtccat tcgaatttta ccgtgtttag caagggcgaa aagtttgcat 3060

cttgatgatt tagcttgact atgcgattgc tttcctggac ccgtgcagct ggatcccggt 3120

acgcgccgcc acggacgacg gcgtcgtcgg cgttgggccg ctgtcggacc gcgtgcacgt 3180

ggtgtacagc ctgtccaagg acctgggcct cccggggttc cgcgtgggcg ccatctactc 3240

gtccaacgcc ggcgtggtct ccgcggccac caagatgtcg agcttcggcc tggtgtcgtc 3300

ccagacgcag cacctcctgg cgtcgctcct gggcgacagg gacttcacgc ggaggtacat 3360

cgcggagaac acgcggcgga tcagggagcg gcgcgagcag ctggcggagg gcctggcggc 3420

cgtgggcatc gagtgcctgg agagcaacgc ggggctcttc tgctgggtca acatgcggcg 3480

cctgatgcgg agccggtcgt tcgagggcga gatggagctg tggaagaggg tggtcttcga 3540

ggtggggctc aacatctccc cgggctcctc ctgccactgc cgggagcccg gctggttccg 3600

cgtctgctaa agggcgaatt ccagcacact ggcggccgtt actagtggat ccgagct 3657

<210> SEQ ID NO 6
<211> LENGTH: 6772
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: See Table 1 of specification

<400> SEQUENCE: 6

gtgcagcgtg acccggtcgt gcccctctct agagataatg agcattgcat gtctaagtta 60

taaaaaatta ccacatattt tttttgtcac acttgtttga agtgcagttt atctatcttt 120

atacatatat ttaaacttta ctctacgaat aatataatct atagtactac aataatatca 180

gtgttttaga gaatcatata aatgaacagt tagacatggt ctaaaggaca attgagtatt 240

ttgacaacag gactctacag ttttatcttt ttagtgtgca tgtgttctcc tttttttttg 300

caaatagctt cacctatata atacttcatc cattttatta gtacatccat ttagggttta 360

gggttaatgg tttttataga ctaatttttt tagtacatct attttattct attttagcct 420

ctaaattaag aaaactaaaa ctctatttta gtttttttat ttaataattt agatataaaa 480

tagaataaaa taaagtgact aaaaattaaa caaataccct ttaagaaatt aaaaaaacta 540

aggaaacatt tttcttgttt cgagtagata atgccagcct gttaaacgcc gtcgacgagt 600

ctaacggaca ccaaccagcg aaccagcagc gtcgcgtcgg gccaagcgaa gcagacggca 660

cggcatctct gtcgctgcct ctggacccct ctcgagagtt ccgctccacc gttggacttg 720

ctccgctgtc ggcatccaga aattgcgtgg cggagcggca gacgtgagcc ggcacggcag 780

gcggcctcct cctcctctca cggcaccggc agctacgggg gattcctttc ccaccgctcc 840

ttcgctttcc cttcctcgcc cgccgtaata aatagacacc ccctccacac cctctttccc 900

caacctcgtg ttgttcggag cgcacacaca cacaaccaga tctcccccaa atccacccgt 960

cggcacctcc gcttcaaggt acgccgctcg tcctcccccc ccccccctct taccttctct 1020

agatcggcgt tccggtccat gcatggttag ggcccggtag ttctacttct gttcatgttt 1080

gtgttagatc cgtgtttgtg ttagatccgt gctgctagcg ttcgtacacg gatgcgacct 1140

-continued

gtacgtcaga cacgttctga ttgctaactt gccagtgttt ctctttgggg aatcctggga 1200

tggctctagc cgttccgcag acgggatcga tttcatgatt ttttttgttt cgttgcatag 1260

ggtttggttt gccctttcc tttatttcaa tatatgccgt gcacttgttt gtcgggtcat 1320

cttttcatgc ttttttttgt cttggttgtg atgatgtggt ctggttgggc ggtcgttcta 1380

gatcggagta gaattctgtt tcaaactacc tggtggattt attaattttg gatctgtatg 1440

tgtgtgccat acatattcat agttacgaat tgaagatgat ggatggaaat atcgatctag 1500

gataggtata catgttgatg cgggttttac tgatgcatat acagagatgc tttttgttcg 1560

cttggttgtg atgatgtggt gtggttgggc ggtcgttcat tcgttctaga tcggagtaga 1620

atactgtttc aaactacctg gtgtatttat taattttgga actgtatgtg tgtgtcatac 1680

atcttcatag ttacgagttt aagatggatg gaaatatcga tctaggatag gtatacatgt 1740

tgatgtgggt tttactgatg catatacatg atggcatatg cagcatctat tcatatgctc 1800

taaccttgag tacctatcta ttataataaa caagtatgtt ttataattat tttgatcttg 1860

atatacttgg atgatggcat atgcagcagc tatatgtgga tttttttagc cctgccttca 1920

tacgctattt atttgcttgg tactgtttct tttgtcgatg ctcaccctgt tgtttggtgt 1980

tacttctgca ggtcgacttt aacttagcct aggatccact agtaacggcc gccagtgtgc 2040

tggaattcgc cctttagcag acgcggaacc agccgggctc ccggcagtgg caggaggagc 2100

ccggggagat gttgagcccc acctcgaaga ccaccctctt ccacagctcc atctcgccct 2160

cgaacgaccg gctccgcatc aggcgccgca tgttgaccca gcagaagagc cccgcgttgc 2220

tctccaggca ctcgatgccc acggccgcca ggccctccgc cagctgctcg cgccgctccc 2280

tgatccgccg cgtgttctcc gcgatgtacc tccgcgtgaa gtccctgtcg cccaggagcg 2340

acgccaggag gtgctgcgtc tgggacgaca ccaggccgaa gctcgacatc ttggtggccg 2400

cggagaccac gccggcgttg gacgagtaga tggcgcccac gcggaacccc gggaggccca 2460

ggtccttgga caggctgtac accacgtgca cgcggtccga cagcggccca acgccgacga 2520

cgccgtcgtc cgtggcggcg cgcgcggcca ccacctgcag tcgacgtgca aaggtccgcc 2580

ttgtttctcc tctgtctctt gatctgacta atcttggttt atgattcgtt gagtaatttt 2640

ggggaaagct tcgtccacag ttttttttcg atgaacagtg ccgcagtggc gctgatcttg 2700

tatgctatcc tgcaatcgtg gtgaacttat ttcttttata tcctttactc ccatgaaaag 2760

gctagtaatc tttctcgatg taacatcgtc cagcactgct attaccgtgt ggtccatccg 2820

acagtctggc tgaacacatc atacgatcta tggagcaaaa atctatcttc cctgttcttt 2880

aatgaaggac gtcattttca ttagtatgat ctaggaatgt tgcaacttgc aaggaggcgt 2940

ttctttcttt gaatttaact aactcgttga gtggccctgt ttctcggacg taaggccttt 3000

gctgctccac acatgtccat tcgaatttta ccgtgtttag caagggcgaa aagtttgcat 3060

cttgatgatt tagcttgact atgcgattgc tttcctggac ccgtgcagct ggatcccggt 3120

acgcgccgcc acggacgacg gcgtcgtcgg cgttgggccg ctgtcggacc gcgtgcacgt 3180

ggtgtacagc ctgtccaagg acctgggcct cccggggttc cgcgtgggcg ccatctactc 3240

gtccaacgcc ggcgtggtct ccgcggccac caagatgtcg agcttcggcc tggtgtcgtc 3300

ccagacgcag cacctcctgg cgtcgctcct gggcgacagg gacttcacgc ggaggtacat 3360

cgcggagaac acgcggcgga tcagggagcg gcgcgagcag ctggcggagg gcctggcggc 3420

-continued

cgtgggcatc gagtgcctgg agagcaacgc ggggctcttc tgctgggtca acatgcggcg 3480

cctgatgcgg agccggtcgt tcgagggcga gatggagctg tggaagaggg tggtcttcga 3540

ggtggggctc aacatctccc cgggctcctc ctgccactgc cgggagcccg gctggttccg 3600

cgtctgctaa agggcgaatt ccagcacact ggcggccgtt actagtggat ccgagctcga 3660

attccggtcc gggtcacccg gtccgggcct agaaggccga tctcccgggc acccagcttt 3720

cttgtacaaa gtggtgatat cggaccgatt aaactttaat tcggtccgat gcatgtatac 3780

gaagttccta ttccgaagtt cctattctac atagagtata ggaacttcac ctggtggcgc 3840

cgctagtgga tcccccgggc tgcagtgcag cgtgacccgg tcgtgcccct ctctagagat 3900

aatgagcatt gcatgtctaa gttataaaaa attaccacat attttttttg tcacacttgt 3960

ttgaagtgca gtttatctat ctttatacat atatttaaac tttactctac gaataatata 4020

atctatagta ctacaataat atcagtgttt tagagaatca tataaatgaa cagttagaca 4080

tggtctaaag gacaattgag tattttgaca acaggactct acagttttat ctttttagtg 4140

tgcatgtgtt ctccttttt tttgcaaata gcttcaccta tataatactt catccatttt 4200

attagtacat ccatttaggg tttagggtta atggttttta tagactaatt tttttagtac 4260

atctatttta ttctatttta gcctctaaat taagaaaact aaaactctat tttagttttt 4320

ttatttaata atttagatat aaaatagaat aaaataaagt gactaaaaat taaacaaata 4380

ccctttaaga aattaaaaaa actaaggaaa catttttctt gtttcgagta gataatgcca 4440

gcctgttaaa cgccgtcgac gagtctaacg gacaccaacc agcgaaccag cagcgtcgcg 4500

tcgggccaag cgaagcagac ggcacggcat ctctgtcgct gcctctggac ccctctcgag 4560

agttccgctc caccgttgga cttgctccgc tgtcggcatc cagaaattgc gtggcggagc 4620

ggcagacgtg agccggcacg gcaggcggcc tcctcctcct ctcacggcac cggcagctac 4680

gggggattcc tttcccaccg ctccttcgct ttcccttcct cgcccgccgt aataaatага 4740

caccccctcc acaccctctt tccccaacct cgtgttgttc ggagcgcaca cacacacaac 4800

cagatctccc ccaaatccac ccgtcggcac ctccgcttca aggtacgccg ctcgtcctcc 4860

ccccccccc tctctacctt ctctagatcg gcgttccggt ccatgcatgg ttagggcccg 4920

gtagttctac ttctgttcat gtttgtgtta gatccgtgtt tgtgttagat ccgtgctgct 4980

agcgttcgta cacggatgcg acctgtacgt cagacacgtt ctgattgcta acttgccagt 5040

gtttctcttt ggggaatcct gggatggctc tagccgttcc gcagacggga tcgatttcat 5100

gatttttttt gtttcgttgc atagggtttg gtttgccctt ttcctttatt tcaatatatg 5160

ccgtgcactt gtttgtcggg tcatcttttc atgcttttтt ttgtcttggt tgtgatgatg 5220

tggtctggtt gggcggtcgt tctagatcgg agtagaattc tgtttcaaac tacctggtgg 5280

atttattaat tttggatctg tatgtgtgtg ccatacatat tcatagttac gaattgaaga 5340

tgatggatgg aaatatcgat ctaggatagg tatacatgtt gatgcgggtt ttactgatgc 5400

atatacagag atgctttttg ttcgcttggt tgtgatgatg tggtgtggtt gggcggtcgt 5460

tcattcgttc tagatcggag tagaatactg tttcaaacta cctggtgtat ttattaattt 5520

tggaactgta tgtgtgtgtc atacatcttc atagttacga gtttaagatg gatggaaata 5580

tcgatctagg ataggtatac atgttgatgt gggttttact gatgcatata catgatggca 5640

tatgcagcat ctattcatat gctctaacct tgagtaccta tctattataa taaacaagta 5700

-continued

tgttttataa ttattttgat cttgatatac ttggatgatg gcatatgcag cagctatatg 5760

tggatttttt tagccctgcc ttcatacgct atttatttgc ttggtactgt ttcttttgtc 5820

gatgctcacc ctgttgtttg gtgttacttc tgcaggtcga ctttaactta gcctaggatc 5880

cacacgacac catgtccccc gagcgccgcc ccgtcgagat ccgcccggcc accgccgccg 5940

acatggccgc cgtgtgcgac atcgtgaacc actacatcga gacctccacc gtgaacttcc 6000

gcaccgagcc gcagaccccg caggagtgga tcgacgacct ggagcgcctc caggaccgct 6060

acccgtggct cgtggccgag gtggagggcg tggtggccgg catcgcctac gccggcccgt 6120

ggaaggcccg caacgcctac gactggaccg tggagtccac cgtgtacgtg tcccaccgcc 6180

accagcgcct cggcctcggc tccaccctct acacccacct cctcaagagc atggaggccc 6240

agggcttcaa gtccgtggtg gccgtgatcg gcctcccgaa cgacccgtcc gtgcgcctcc 6300

acgaggccct cggctacacc gcccgcggca ccctccgcgc cgccggctac aagcacggcg 6360

gctggcacga cgtcggcttc tggcagcgcg acttcgagct gccggccccg ccgcgcccgg 6420

tgcgcccggt gacgcagatc tgagtcgaaa cctagacttg tccatcttct ggattggcca 6480

acttaattaa tgtatgaaat aaaaggatgc acacatagtg acatgctaat cactataatg 6540

tgggcatcaa agttgtgtgt tatgtgtaat tactagttat ctgaataaaa gagaaagaga 6600

tcatccatat ttcttatcct aaatgaatgt cacgtgtctt tataattctt tgatgaacca 6660

gatgcatttc attaaccaaa tccatataca tataaatatt aatcatatat aattaatatc 6720

aattgggtta gcaaaacaaa tctagtctag gtgtgttttg cgaattgcgg cc 6772

<210> SEQ ID NO 7
<211> LENGTH: 8350
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: See Table 1 of specification

<400> SEQUENCE: 7

gtttacccgc caatatatcc tgtcaaacac tgatagttta aactgaaggc gggaaacgac 60

aatctgatca tgagcggaga attaagggag tcacgttatg acccccgccg atgacgcggg 120

acaagccgtt ttacgtttgg aactgacaga accgcaacgt tgaaggagcc actcagcaag 180

ctggtacgat tgtaatacga ctcactatag ggcgaattga gcgctgttta aacgctcttc 240

aactggaaga gcggttacta ccggctggat ggcggggcct tgatcgtgca ccgccggcgt 300

ccggataagt gactagggtc acgtgaccct agtcacttat cgagctagtt accctatgag 360

gtgacatgaa gcgctcacgg ttactatgac ggttagcttc acgactgttg gtggcagtag 420

cgtacgactt agctatagtt ccggtagatc tgaagttcct attccgaagt tcctattctt 480

caaaaggtat aggaacttcc tcgaattgtt gtggtggggt atagaggttt gatataggtg 540

gaactgctgt agagcgtgga gatatagggg gaaagagaac gctgatgtga caagtgagtg 600

agatataggg ggagaaattt agggggaacg ccgaacacag tctaaagaag cttgggaccc 660

aaagcactct gttcgggggt ttttttttt gtctttcaac tttttgctgt aatgttattc 720

aaaataagaa aagcacttgg catggctaag aaatagagtt caacaactga acagtacagt 780

gtattatcaa tggcataaaa aacaaccctt acagcattgc cgtattttat tgatcaaaca 840

ttcaactcaa cactgacgag tggtcttcca ccgatcaacg gactaatgct gctttgtcag 900

-continued

atcaccggtt aagtgactag ggtcacgtga ccctagtcac ttaggttacc agagctggtc 960

acctttgtcc accaagatgg aactgcggcc gctcattaat taagtcaggc gcgcctctag 1020

ttgaagacac gttcatgtct tcatcgtaag aagacactca gtagtcttcg gccagaatgg 1080

ccatctggat tcagcaggcc tagaaggcca tttaaatcct gaggatctgg tcttcctaag 1140

gacccgggat atcacaagtt tgtacaaaaa agcaggctcc ggccagagtt acccggaccg 1200

aagcttgcat gcctgcagtg cagcgtgacc cggtcgtgcc cctctctaga gataatgagc 1260

attgcatgtc taagttataa aaaattacca catatttttt ttgtcacact tgtttgaagt 1320

gcagtttatc tatctttata catatattta aactttactc tacgaataat ataatctata 1380

gtactacaat aatatcagtg ttttagagaa tcatataaat gaacagttag acatggtcta 1440

aaggacaatt gagtattttg acaacaggac tctacagttt tatcttttta gtgtgcatgt 1500

gttctccttt ttttttgcaa atagcttcac ctatataata cttcatccat tttattagta 1560

catccattta gggtttaggg ttaatggttt ttatagacta atttttttag tacatctatt 1620

ttattctatt ttagcctcta aattaagaaa actaaaactc tattttagtt tttttattta 1680

ataatttaga tataaaatag aataaaataa agtgactaaa aattaaacaa atacccttta 1740

agaaattaaa aaaactaagg aaacattttt cttgtttcga gtagataatg ccagcctgtt 1800

aaacgccgtc gacgagtcta acggacacca accagcgaac cagcagcgtc gcgtcgggcc 1860

aagcgaagca gacggcacgg catctctgtc gctgcctctg gacccctctc gagagttccg 1920

ctccaccgtt ggacttgctc cgctgtcggc atccagaaat tgcgtggcgg agcggcagac 1980

gtgagccggc acggcaggcg gcctcctcct cctctcacgg caccggcagc tacgggggat 2040

tcctttccca ccgctccttc gctttccctt cctcgcccgc cgtaataaat agacacccca 2100

tccacaccct ctttccccaa cctcgtgttg ttcggagcgc acacacacac aaccagatct 2160

cccccaaatc cacccgtcgg cacctccgct tcaaggtacg ccgctcgtcc tcccccccca 2220

ccctctctac cttctctaga tcggcgttcc ggtccatgca tggttagggc ccggtagttc 2280

tacttctgtt catgtttgtg ttagatccgt gtttgtgtta gatccgtgct gctagcgttc 2340

gtacacggat gcgacctgta cgtcagacac gttctgattg ctaacttgcc agtgtttctc 2400

tttggggaat cctgggatgg ctctagccgt tccgcagacg ggatcgattt catgattttt 2460

tttgtttcgt tgcatagggt ttggtttgcc cttttccttt atttcaatat atgccgtgca 2520

cttgtttgtc gggtcatctt ttcatgcttt tttttgtctt ggttgtgatg atgtggtctg 2580

gttgggcggt cgttctagat cggagtagaa ttctgtttca aactacctgg tggatttatt 2640

aattttggat ctgtatgtgt gtgccataca tattcatagt tacgaattga agatgatgga 2700

tggaaatatc gatctaggat aggtatacat gttgatgcgg gttttactga tgcatataca 2760

gagatgcttt ttgttcgctt ggttgtgatg atgtggtgtg gttgggcggt cgttcattcg 2820

ttctagatcg gagtagaata ctgtttcaaa ctacctggtg tatttattaa ttttggaact 2880

gtatgtgtgt gtcatacatc ttcatagtta cgagtttaag atggatggaa atatcgatct 2940

aggataggta tacatgttga tgtgggtttt actgatgcat atacatgatg gcatatgcag 3000

catctattca tatgctctaa ccttgagtac ctatctatta taataaacaa gtatgtttta 3060

taattatttt gatcttgata tacttggatg atggcatatg cagcagctat atgtggattt 3120

ttttagccct gccttcatac gctatttatt tgcttggtac tgtttctttt gtcgatgctc 3180

-continued

accctgttgt ttggtgttac ttctgcaggt cgactttaac ttagcctagg atccactagt 3240

aacggccgcc agtgtgctgg aattcgccct ttagcagacg cggaaccagc cgggctcccg 3300

gcagtggcag gaggagcccg gggagatgtt gagccccacc tcgaagacca ccctcttcca 3360

cagctccatc tcgccctcga acgaccggct ccgcatcagg cgccgcatgt tgacccagca 3420

gaagagcccc gcgttgctct ccaggcactc gatgcccacg gccgccaggc cctccgccag 3480

ctgctcgcgc cgctccctga tccgccgcgt gttctccgcg atgtacctcc gcgtgaagtc 3540

cctgtcgccc aggagcgacg ccaggaggtg ctgcgtctgg gacgacacca ggccgaagct 3600

cgacatcttg gtggccgcgg agaccacgcc ggcgttggac gagtagatgg cgcccacgcg 3660

gaaccccggg aggcccaggt ccttggacag gctgtacacc acgtgcacgc ggtccgacag 3720

cggcccaacg ccgacgacgc cgtcgtccgt ggcggcgcgc gcggccacca cctgcagtcg 3780

acgtgcaaag gtccgccttg tttctcctct gtctcttgat ctgactaatc ttggtttatg 3840

attcgttgag taattttggg gaaagcttcg tccacagttt tttttcgatg aacagtgccg 3900

cagtggcgct gatcttgtat gctatcctgc aatcgtggtg aacttatttc ttttatatcc 3960

tttactccca tgaaaaggct agtaatcttt ctcgatgtaa catcgtccag cactgctatt 4020

accgtgtggt ccatccgaca gtctggctga acacatcata cgatctatgg agcaaaaatc 4080

tatcttccct gttctttaat gaaggacgtc attttcatta gtatgatcta ggaatgttgc 4140

aacttgcaag gaggcgtttc tttctttgaa tttaactaac tcgttgagtg gccctgtttc 4200

tcggacgtaa ggcctttgct gctccacaca tgtccattcg aattttaccg tgtttagcaa 4260

gggcgaaaag tttgcatctt gatgatttag cttgactatg cgattgcttt cctggacccg 4320

tgcagctgga tcccggtacg cgccgccacg gacgacggcg tcgtcggcgt tgggccgctg 4380

tcggaccgcg tgcacgtggt gtacagcctg tccaaggacc tgggcctccc ggggttccgc 4440

gtgggcgcca tctactcgtc caacgccggc gtggtctccg cggccaccaa gatgtcgagc 4500

ttcggcctgg tgtcgtccca gacgcagcac ctcctggcgt cgctcctggg cgacagggac 4560

ttcacgcgga ggtacatcgc ggagaacacg cggcggatca gggagcggcg cgagcagctg 4620

gcggagggcc tggcggccgt gggcatcgag tgcctggaga gcaacgcggg gctcttctgc 4680

tgggtcaaca tgcggcgcct gatgcggagc cggtcgttcg agggcgagat ggagctgtgg 4740

aagagggtgg tcttcgaggt ggggctcaac atctccccgg gctcctcctg ccactgccgg 4800

gagcccggct ggttccgcgt ctgctaaagg gcgaattcca gcacactggc ggccgttact 4860

agtggatccg agctcgaatt ccggtccggg tcacccggtc cgggcctaga aggccgatct 4920

cccgggcacc cagctttctt gtacaaagtg gtgatatcgg accgattaaa ctttaattcg 4980

gtccgatgca tgtatacgaa gttcctattc cgaagttcct attctacata gagtatagga 5040

acttcacctg gtggcgccgc tagtggatcc cccgggctgc agtgcagcgt gacccggtcg 5100

tgcccctctc tagagataat gagcattgca tgtctaagtt ataaaaaatt accacatatt 5160

ttttttgtca cacttgtttg aagtgcagtt tatctatctt tatacatata tttaaacttt 5220

actctacgaa taatataatc tatagtacta caataatatc agtgttttag agaatcatat 5280

aaatgaacag ttagacatgg tctaaaggac aattgagtat tttgacaaca ggactctaca 5340

gttttatctt tttagtgtgc atgtgttctc ctttttttttt gcaaatagct tcacctatat 5400

aatacttcat ccattttatt agtacatcca tttagggttt agggttaatg gtttttatag 5460

-continued

actaattttt ttagtacatc tattttattc tattttagcc tctaaattaa gaaaactaaa 5520

actctatttt agtttttta tttaataatt tagatataaa atagaataaa ataaagtgac 5580

taaaaattaa acaaatacc tttaagaaat taaaaaaact aaggaaacat ttttcttgtt 5640

tcgagtagat aatgccagcc tgttaaacgc cgtcgacgag tctaacggac accaaccagc 5700

gaaccagcag cgtcgcgtcg ggccaagcga agcagacggc acggcatctc tgtcgctgcc 5760

tctggacccc tctcgagagt tccgctccac cgttggactt gctccgctgt cggcatccag 5820

aaattgcgtg gcggagcggc agacgtgagc cggcacggca ggcggcctcc tcctcctctc 5880

acggcaccgg cagctacggg ggattccttt cccaccgctc cttcgctttc ccttcctcgc 5940

ccgccgtaat aaatagacac cccctccaca ccctctttcc ccaacctcgt gttgttcgga 6000

gcgcacacac acacaaccag atctcccca aatccacccg tcggcacctc cgcttcaagg 6060

tacgccgctc gtcctccccc ccccccctct ctaccttctc tagatcggcg ttccggtcca 6120

tgcatggtta gggcccggta gttctacttc tgttcatgtt tgtgttagat ccgtgtttgt 6180

gttagatccg tgctgctagc gttcgtacac ggatgcgacc tgtacgtcag acacgttctg 6240

attgctaact tgccagtgtt tctctttggg gaatcctggg atggctctag ccgttccgca 6300

gacgggatcg atttcatgat ttttttgtt tcgttgcata gggtttggtt tgccctttc 6360

ctttatttca atatatgccg tgcacttgtt tgtcgggtca tcttttcatg ctttttttg 6420

tcttggttgt gatgatgtgg tctggttggg cggtcgttct agatcggagt agaattctgt 6480

ttcaaactac ctggtggatt tattaatttt ggatctgtat gtgtgtgcca tacatattca 6540

tagttacgaa ttgaagatga tggatggaaa tatcgatcta ggataggtat acatgttgat 6600

gcgggtttta ctgatgcata tacagagatg ctttttgttc gcttggttgt gatgatgtgg 6660

tgtggttggg cggtcgttca ttcgttctag atcggagtag aatactgttt caaactacct 6720

ggtgtattta ttaattttgg aactgtatgt gtgtgtcata catcttcata gttacgagtt 6780

taagatggat ggaaatatcg atctaggata ggtatacatg ttgatgtggg ttttactgat 6840

gcatatacat gatggcatat gcagcatcta ttcatatgct ctaaccttga gtacctatct 6900

attataataa acaagtatgt tttataatta ttttgatctt gatatacttg gatgatggca 6960

tatgcagcag ctatatgtgg attttttag ccctgccttc atacgctatt tatttgcttg 7020

gtactgtttc ttttgtcgat gctcaccctg ttgtttggtg ttacttctgc aggtcgactt 7080

taacttagcc taggatccac acgacaccat gtcccccgag cgccgccccg tcgagatccg 7140

cccggccacc gccgccgaca tggccgccgt gtgcgacatc gtgaaccact acatcgagac 7200

ctccaccgtg aacttccgca ccgagccgca gaccccgcag gagtggatcg acgacctgga 7260

gcgcctccag gaccgctacc cgtggctcgt ggccgaggtg gagggcgtgg tggccggcat 7320

cgcctacgcc ggcccgtgga aggcccgcaa cgcctacgac tggaccgtgg agtccaccgt 7380

gtacgtgtcc caccgccacc agcgcctcgg cctcggctcc accctctaca cccacctcct 7440

caagagcatg gaggcccagg gcttcaagtc cgtggtggcc gtgatcggcc tcccgaacga 7500

cccgtccgtg cgcctccacg aggccctcgg ctacaccgcc cgcggcaccc tccgcgccgc 7560

cggctacaag cacggcggct ggcacgacgt cggcttctgg cagcgcgact tcgagctgcc 7620

ggccccgccg cgcccggtgc gcccggtgac gcagatctga gtcgaaacct agacttgtcc 7680

atcttctgga ttggccaact taattaatgt atgaaataaa aggatgcaca catagtgaca 7740

-continued

tgctaatcac tataatgtgg gcatcaaagt tgtgtgttat gtgtaattac tagttatctg 7800

aataaaagag aaagagatca tccatatttc ttatcctaaa tgaatgtcac gtgtctttat 7860

aattctttga tgaaccagat gcatttcatt aaccaaatcc atatacatat aaatattaat 7920

catatataat taatatcaat tgggttagca aaacaaatct agtctaggtg tgttttgcga 7980

attgcggccg ctctagcgta tacgaagttc ctattccgaa gttcctattc tctagaaagt 8040

ataggaactt ctgattccga tgacttcgta ggttcctagc tcaagccgct cgtgtccaag 8100

cgtcacttac gattagctaa tgattacggc atctaggacc gactagtaag tgactagggt 8160

cacgtgaccc tagtcactta tacgtagaat taattcattc cgattaatcg tggcctcttg 8220

ctcttcagga tgaagagcta tgtttaaacg tgcaagcgct actagacaat tcagtacatt 8280

aaaaacgtcc gcaatgtgtt attaagttgt ctaagcgtca atttgtttac accacaatat 8340

atcctgccac 8350

<210> SEQ ID NO 8
<211> LENGTH: 1424
<212> TYPE: DNA
<213> ORGANISM: Sorghum bicolor
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(1424)
<223> OTHER INFORMATION: sorghum PEP carboxylase promoter

<400> SEQUENCE: 8

ggacatgtaa taaggagtta ggagatgtgg tgtggtacta aatgcaaggt caaaattcga 60

tgctttttcc gtgctcaact attaactagt actcattatt acctaatttt cacttgtgat 120

gacaattaat gcatcgatcc acaattcagt aaatactttc atttaagcat atgtatagta 180

ttatacattt ccaattcttc ttttttgtgt ggagatccac gacgatgcaa gttgctcctc 240

ccaacccaaa tccacctctc tcttaaatcc gcatatcttc accaccacca gctgctacac 300

atcgtattgt ccaaatctgt gtcggcttga cccagtgatg tgcgcgctag atttggcagc 360

gcctgaatgc tgtgcagcca cctgtatggt gcccttggta gagtaacaac acccttatcc 420

ctacggcagc catgtatgac ccttatccct acggcagcca tgtataccaa tacctttctt 480

tgaaccacaa aattatagtc catatcctta accacaagtt catttttgt ttcccggtct 540

cctaaggaaa ttaagttctg tttccacagt ttacatggat ataggacatc tatgttccta 600

acattaacat tactggataa caggcaccct ctcctccaca ccctgcaaag ccttcctcca 660

gcgccatgca tcctccgttg ctaacagaca cctctctcca catcgcgtgc aagcaaacct 720

ccaaattcta ccgatcccca gaatccggcc ttgactgcaa acagacaccc ctctccccat 780

cctgcaaacc catcagccaa ccgaataaca caagaaggca ggtgagcagt gacaaagcac 840

gtcaacagca gcaaagccaa gccaaaaacg atccaggagc aaggtgcggc cgcagctctc 900

ccggtcccct ttgcggttac cactagctaa gaatgaagat ggtactctaa atggatactt 960

gcgcggtttt tctctagtct aacttaataa actaaataaa caatttcttt cttatttttt 1020

taatttagtt cgtttagtta gactagagaa gaaccacgag gagttatttg aagcgtcgtc 1080

cccatcctta ccactagcta gcactagcag acacccctct ccacgtcctg caaacaggca 1140

attagccagc ggaataacac aagcaggcaa gtgcgcagtg acaaagtacg tccacagcag 1200

cgatcccagc caaaagcagc gtagccacag ccgcgcgcag ctctcggcta cccttaccgc 1260

-continued

cgatcacatg catgcctttc caatcccgcg tgcacacgcc gaccacacac tcgccaactc 1320

cccatcccta tttgaagcca ccggccggcg ccctgcattg atcaatcaac tcgcagcaga 1380

ggagcagcac gagcaacacg ccgcgccgcg ctccaaccat ctcc 1424

<210> SEQ ID NO 9
<211> LENGTH: 2765
<212> TYPE: DNA
<213> ORGANISM: Zea mays
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(2765)
<223> OTHER INFORMATION: genomic ACS3 sequence
<220> FEATURE:
<221> NAME/KEY: 5'UTR
<222> LOCATION: (214)...(372)
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (373)...(714)
<220> FEATURE:
<221> NAME/KEY: intron
<222> LOCATION: (715)...(818)
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (819)...(979)
<220> FEATURE:
<221> NAME/KEY: intron
<222> LOCATION: (980)...(1106)
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1107)...(1992)
<220> FEATURE:
<221> NAME/KEY: 3'UTR
<222> LOCATION: (1993)...(2203)

<400> SEQUENCE: 9

tagtagacta aagctcccgg ctttcccttt attgttacta ttattatatc gtttgatttg 60

cagcggacag tctggtcgtt tccgtctgct ctggaccctg ccttcaattc tccgcctttt 120

gacatcttgg gtggtttcac acgggtatat atacttgact cgcctatata tgccaccccc 180

cacacacttg gccagatcga ccaccgcatc acacagcagc agcctcttgt gctcctctct 240

gtcctttgct tgctcatccc tcgacctcga gcggcggcgg cggtcactgc actgcgtgtg 300

cgtatccatc catcttgcag gcatcgtcgt ctgcaccgcg cgcttgtgcg tgagccaagg 360

gcggcagagg cgatgggtgg caagctgttg ctgggcgcga gccagagccg ccacgcgcac 420

gcggtggcgt cgcctcccct gtcaaaggtg gccacttccg gcctccacgg cgaggactcg 480

ccctacttcg ccgggtggaa agcctacgac gagaacccct acgacgccgt ctccaacccc 540

ggcggcgtca ttcagatggg cctcgccgag aaccaggtgt ccttcgacct cctcgagggg 600

tacctcaggg accacccgga ggccgcgggc tggggcggct ccggctccgg cgtcgccagc 660

ttcagggaca acgcgctgtt ccaggactac cacggcctca aggccttcag aaaggtgcgt 720

ggtgaccccg agcgcctgct ctgctcgtct tgttcggtgt cctggataga ccctgcctgg 780

acaagtgcaa cgtactgacg gcgtcatttt gcttgcaggc gatggccaac ttcatggaga 840

aggttagggg cggcaaggcc cggtttgacc ccgaccgcat cgtgctcacc gccggcgcca 900

cggcagctaa cgagctgctc acgttcgtcc tggccaaccc gggagacgcg ctgctgatcc 960

ctactcctta ctatcctggg taagcggagc gctaagaagc atccagcatg ctgcatgcat 1020

gcacggaccc ccggcgctat gctactgtta caccgcaaca gtgctctaca cagacagaca 1080

gacaaactga catgctctgc ttgcagtttc gacagagacc tgcggtggag gacgggggtg 1140

-continued

aacatcgtgc cggtgcactg cgacagcgcc aacgggttcc aggtcacggc cgccgcgctc 1200

caggcggcgt acgaggaggc cgaggcagcg gggacgcgcg tccgcgccgt cctgctcacc 1260

aacccgtcca acccgctggg caccaccgtg acgcggccgg ccctcgagga cgtgctcgac 1320

ttcgtggccc gcaacaacat ccacctcatc tccgacgaga tatactccgg ctcggtcttc 1380

gcggcgccgg acctggtcag cgtggcggag ctggtcgagt cccgcggcga ccccggcgtc 1440

gcggagcgcg tccacatcgt gtacagcctg tccaaggacc tgggcctccc ggggttccgc 1500

gtcggcgtcg tgtactcgta caacgacgcc gtggtcaccg cggcgcgccg catgtccagc 1560

ttcacgctcg tgtcgtcgca gacgcagaag acgctcgccg ccatgctctc ggacgccggg 1620

ttcgcggacg cctacgtccg caccaaccgc cagcgcctcc gggcgcggca cgaccacgtc 1680

gtcgccgggc tggcccgcgc gggcgtgccg tgcctccgcg gcaacgccgg gctgttcgtg 1740

tggatggaca tgaggcggct gctcggcgag gccaccaccg tcgccggcga gctccgcctg 1800

tgggaccgga tgctgcggga ggcgaagctc aacatctcgc cgggctcgtc gtgccattgc 1860

tcggagcctg gctggttcag ggtgtgcttc gccaacatga gcctggacac gctggatgtt 1920

gcactcgcta ggatgagccg cttcgtagac acgtggaaca aggaaacgac agcgtcgacg 1980

cagcagcact agcagcagca gcagcatacg aagtaaattt tttggagggt aaattacgtc 2040

attggacaga ttaaatcaca gagtagttat acaggggat tcttttatgg tttttcgatt 2100

gatggtaaca tcgattttgt aacaataact atcgcctctc agatggagga gggacacata 2160

tatgtatgta tttataaaaa ttcttacttt ggcccaagca aaagcgtctc cgctacacat 2220

tcagtattat tgttttgctt cgtttttccc tcctgagttg tggcaaacaa acacagatga 2280

cgtatgtttg ctcattcatt catatattta tatctgcctg gaagcgaacc taaattagca 2340

acaagagaga acatgtccat tccgagtact gagaataagt gcagcagaat gaatgctggt 2400

tgttggattt aaattagatg gatgttgttt gtcgtcagaa aatggagaga gagaaatcag 2460

ttagcgaatc cctttctcgt tttatactgc tggttttcta tacttttgaa gaaaaaaaac 2520

tgttttgctg aggtgttcga tgttgtaaat tactgattga cttcatgtga ttgcgtaaca 2580

tgttactgga cggaggttta cttctataca tgagtagtgt cataccaaac caaaaaaaaa 2640

agggaatcga aagtcttgat agcggacgtg ttgattaaag aaaaagaaac cgagttccaa 2700

atgtcaaccc ttccttaagc cggtagtaag ctttgtcaac cagctaaata agggaacgca 2760

tatcc 2765

<210> SEQ ID NO 10
<211> LENGTH: 1759
<212> TYPE: DNA
<213> ORGANISM: Zea mays
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (160)...(1548)
<223> OTHER INFORMATION: ACS3

<400> SEQUENCE: 10

cagcagcagc ctcttgtgct cctctctgtc ctttgcttgc tcatccctcg acctcgagcg 60

gcggcggcgg tcactgcact gcgtgtgcgt atccatccat cttgcaggca tcgtcgtctg 120

caccgcgcgc ttgtgcgtga gccaagggcg gcagaggcg atg ggt ggc aag ctg 174
Met Gly Gly Lys Leu
1 5

-continued

```
ttg ctg ggc gcg agc cag agc cgc cac gcg cac gcg gtg gcg tcg cct      222
Leu Leu Gly Ala Ser Gln Ser Arg His Ala His Ala Val Ala Ser Pro
                10                  15                  20

ccc ctg tca aag gtg gcc act tcc ggc ctc cac ggc gag gac tcg ccc      270
Pro Leu Ser Lys Val Ala Thr Ser Gly Leu His Gly Glu Asp Ser Pro
            25                  30                  35

tac ttc gcc ggg tgg aaa gcc tac gac gag aac ccc tac gac gcc gtc      318
Tyr Phe Ala Gly Trp Lys Ala Tyr Asp Glu Asn Pro Tyr Asp Ala Val
        40                  45                  50

tcc aac ccc ggc ggc gtc att cag atg ggc ctc gcc gag aac cag gtg      366
Ser Asn Pro Gly Gly Val Ile Gln Met Gly Leu Ala Glu Asn Gln Val
    55                  60                  65

tcc ttc gac ctc ctc gag ggg tac ctc agg gac cac ccg gag gcc gcg      414
Ser Phe Asp Leu Leu Glu Gly Tyr Leu Arg Asp His Pro Glu Ala Ala
70                  75                  80                  85

ggc tgg ggc ggc tcc ggc tcc ggc gtc gcc agc ttc agg gac aac gcg      462
Gly Trp Gly Gly Ser Gly Ser Gly Val Ala Ser Phe Arg Asp Asn Ala
                90                  95                  100

ctg ttc cag gac tac cac ggc ctc aag gcc ttc aga aag gcg atg gcc      510
Leu Phe Gln Asp Tyr His Gly Leu Lys Ala Phe Arg Lys Ala Met Ala
            105                 110                 115

aac ttc atg gag aag gtt agg ggc ggc aag gcc cgg ttt gac ccc gac      558
Asn Phe Met Glu Lys Val Arg Gly Gly Lys Ala Arg Phe Asp Pro Asp
        120                 125                 130

cgc atc gtg ctc acc gcc ggc gcc acg gca gct aac gag ctg ctc acg      606
Arg Ile Val Leu Thr Ala Gly Ala Thr Ala Ala Asn Glu Leu Leu Thr
    135                 140                 145

ttc gtc ctg gcc aac ccg gga gac gcg ctg ctg atc cct act cct tac      654
Phe Val Leu Ala Asn Pro Gly Asp Ala Leu Leu Ile Pro Thr Pro Tyr
150                 155                 160                 165

tat cct ggt ttc gac aga gac ctg cgg tgg agg acg ggg gtg aac atc      702
Tyr Pro Gly Phe Asp Arg Asp Leu Arg Trp Arg Thr Gly Val Asn Ile
                170                 175                 180

gtg ccg gtg cac tgc gac agc gcc aac ggg ttc cag gtc acg gcc gcc      750
Val Pro Val His Cys Asp Ser Ala Asn Gly Phe Gln Val Thr Ala Ala
            185                 190                 195

gcg ctc cag gcg gcg tac gag gag gcc gag gca gcg ggg acg cgc gtc      798
Ala Leu Gln Ala Ala Tyr Glu Glu Ala Glu Ala Ala Gly Thr Arg Val
        200                 205                 210

cgc gcc gtc ctg ctc acc aac ccg tcc aac ccg ctg ggc acc acc gtg      846
Arg Ala Val Leu Leu Thr Asn Pro Ser Asn Pro Leu Gly Thr Thr Val
    215                 220                 225

acg cgg ccg gcc ctc gag gac gtg ctc gac ttc gtg gcc cgc aac aac      894
Thr Arg Pro Ala Leu Glu Asp Val Leu Asp Phe Val Ala Arg Asn Asn
230                 235                 240                 245

atc cac ctc atc tcc gac gag ata tac tcc ggc tcg gtc ttc gcg gcg      942
Ile His Leu Ile Ser Asp Glu Ile Tyr Ser Gly Ser Val Phe Ala Ala
                250                 255                 260

ccg gac ctg gtc agc gtg gcg gag ctg gtc gag tcc cgc ggc gac ccc      990
Pro Asp Leu Val Ser Val Ala Glu Leu Val Glu Ser Arg Gly Asp Pro
            265                 270                 275

ggc gtc gcg gag cgc gtc cac atc gtg tac agc ctg tcc aag gac ctg     1038
Gly Val Ala Glu Arg Val His Ile Val Tyr Ser Leu Ser Lys Asp Leu
        280                 285                 290

ggc ctc ccg ggg ttc cgc gtc ggc gtc gtg tac tcg tac aac gac gcc     1086
Gly Leu Pro Gly Phe Arg Val Gly Val Val Tyr Ser Tyr Asn Asp Ala
    295                 300                 305
```

-continued

```
gtg gtc acc gcg gcg cgc cgc atg tcc agc ttc acg ctc gtg tcg tcg     1134
Val Val Thr Ala Ala Arg Arg Met Ser Ser Phe Thr Leu Val Ser Ser
310                 315                 320                 325

cag acg cag aag acg ctc gcc gcc atg ctc tcg gac gcc ggg ttc gcg     1182
Gln Thr Gln Lys Thr Leu Ala Ala Met Leu Ser Asp Ala Gly Phe Ala
                330                 335                 340

gac gcc tac gtc cgc acc aac cgc cag cgc ctc cgg gcg cgg cac gac     1230
Asp Ala Tyr Val Arg Thr Asn Arg Gln Arg Leu Arg Ala Arg His Asp
            345                 350                 355

cac gtc gtc gcc ggg ctg gcc cgc gcg ggc gtg ccg tgc ctc cgc ggc     1278
His Val Val Ala Gly Leu Ala Arg Ala Gly Val Pro Cys Leu Arg Gly
        360                 365                 370

aac gcc ggg ctg ttc gtg tgg atg gac atg agg cgg ctg ctc ggc gag     1326
Asn Ala Gly Leu Phe Val Trp Met Asp Met Arg Arg Leu Leu Gly Glu
    375                 380                 385

gcc acc acc gtc gcc ggc gag ctc cgc ctg tgg gac cgg atg ctg cgg     1374
Ala Thr Thr Val Ala Gly Glu Leu Arg Leu Trp Asp Arg Met Leu Arg
390                 395                 400                 405

gag gcg aag ctc aac atc tcg ccg ggc tcg tcg tgc cat tgc tcg gag     1422
Glu Ala Lys Leu Asn Ile Ser Pro Gly Ser Ser Cys His Cys Ser Glu
                410                 415                 420

cct ggc tgg ttc agg gtg tgc ttc gcc aac atg agc ctg gac acg ctg     1470
Pro Gly Trp Phe Arg Val Cys Phe Ala Asn Met Ser Leu Asp Thr Leu
            425                 430                 435

gat gtt gca ctc gct agg atg agc cgc ttc gta gac acg tgg aac aag     1518
Asp Val Ala Leu Ala Arg Met Ser Arg Phe Val Asp Thr Trp Asn Lys
        440                 445                 450

gaa acg aca gcg tcg acg cag cag cac tag cagcagcagc agcatacgaa       1568
Glu Thr Thr Ala Ser Thr Gln Gln His
    455                 460

gtaaattttt tggagggtaa attacgtcat tggacagatt aaatcacaga gtagttatac   1628

agggggattc ttttatggtt tttcgattga tggtaacatc gattttgtaa caataactat   1688

cgcctctcag atggaggagg gacacatata tgtatgtatt tataaaaatt cttactttgg   1748

cccaagcaaa a                                                       1759
```

<210> SEQ ID NO 11
<211> LENGTH: 462
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 11

```
Met Gly Gly Lys Leu Leu Leu Gly Ala Ser Gln Ser Arg His Ala His
1               5                   10                  15

Ala Val Ala Ser Pro Pro Leu Ser Lys Val Ala Thr Ser Gly Leu His
            20                  25                  30

Gly Glu Asp Ser Pro Tyr Phe Ala Gly Trp Lys Ala Tyr Asp Glu Asn
        35                  40                  45

Pro Tyr Asp Ala Val Ser Asn Pro Gly Gly Val Ile Gln Met Gly Leu
    50                  55                  60

Ala Glu Asn Gln Val Ser Phe Asp Leu Leu Glu Gly Tyr Leu Arg Asp
65                  70                  75                  80

His Pro Glu Ala Ala Gly Trp Gly Gly Ser Gly Ser Gly Val Ala Ser
                85                  90                  95

Phe Arg Asp Asn Ala Leu Phe Gln Asp Tyr His Gly Leu Lys Ala Phe
```

-continued

```
            100                 105                 110

Arg Lys Ala Met Ala Asn Phe Met Glu Lys Val Arg Gly Gly Lys Ala
        115                 120                 125

Arg Phe Asp Pro Asp Arg Ile Val Leu Thr Ala Gly Ala Thr Ala Ala
    130                 135                 140

Asn Glu Leu Leu Thr Phe Val Leu Ala Asn Pro Gly Asp Ala Leu Leu
145                 150                 155                 160

Ile Pro Thr Pro Tyr Tyr Pro Gly Phe Asp Arg Asp Leu Arg Trp Arg
                165                 170                 175

Thr Gly Val Asn Ile Val Pro Val His Cys Asp Ser Ala Asn Gly Phe
            180                 185                 190

Gln Val Thr Ala Ala Ala Leu Gln Ala Ala Tyr Glu Glu Ala Glu Ala
        195                 200                 205

Ala Gly Thr Arg Val Arg Ala Val Leu Leu Thr Asn Pro Ser Asn Pro
    210                 215                 220

Leu Gly Thr Thr Val Thr Arg Pro Ala Leu Glu Asp Val Leu Asp Phe
225                 230                 235                 240

Val Ala Arg Asn Asn Ile His Leu Ile Ser Asp Glu Ile Tyr Ser Gly
                245                 250                 255

Ser Val Phe Ala Ala Pro Asp Leu Val Ser Val Ala Glu Leu Val Glu
            260                 265                 270

Ser Arg Gly Asp Pro Gly Val Ala Glu Arg Val His Ile Val Tyr Ser
        275                 280                 285

Leu Ser Lys Asp Leu Gly Leu Pro Gly Phe Arg Val Gly Val Val Tyr
    290                 295                 300

Ser Tyr Asn Asp Ala Val Val Thr Ala Ala Arg Arg Met Ser Ser Phe
305                 310                 315                 320

Thr Leu Val Ser Ser Gln Thr Gln Lys Thr Leu Ala Ala Met Leu Ser
                325                 330                 335

Asp Ala Gly Phe Ala Asp Ala Tyr Val Arg Thr Asn Arg Gln Arg Leu
            340                 345                 350

Arg Ala Arg His Asp His Val Val Ala Gly Leu Ala Arg Ala Gly Val
        355                 360                 365

Pro Cys Leu Arg Gly Asn Ala Gly Leu Phe Val Trp Met Asp Met Arg
    370                 375                 380

Arg Leu Leu Gly Glu Ala Thr Thr Val Ala Gly Glu Leu Arg Leu Trp
385                 390                 395                 400

Asp Arg Met Leu Arg Glu Ala Lys Leu Asn Ile Ser Pro Gly Ser Ser
                405                 410                 415

Cys His Cys Ser Glu Pro Gly Trp Phe Arg Val Cys Phe Ala Asn Met
            420                 425                 430

Ser Leu Asp Thr Leu Asp Val Ala Leu Ala Arg Met Ser Arg Phe Val
        435                 440                 445

Asp Thr Trp Asn Lys Glu Thr Thr Ala Ser Thr Gln Gln His
    450                 455                 460


<210> SEQ ID NO 12
<211> LENGTH: 1464
<212> TYPE: DNA
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(1461)
```

-continued

<400> SEQUENCE: 12

```
atg gtg agc caa gtg gtc gcc gag gag aag ccg cag ctg ctg tcc aag       48
Met Val Ser Gln Val Val Ala Glu Glu Lys Pro Gln Leu Leu Ser Lys
1               5                  10                  15

aag gcc ggg tgc aac agc cac ggc cag gac tcg tcc tac ttc ctg ggg       96
Lys Ala Gly Cys Asn Ser His Gly Gln Asp Ser Ser Tyr Phe Leu Gly
            20                  25                  30

tgg cag gag tac gag aaa aac ccg ttc gac ccc gtc tcc aac cct tcc      144
Trp Gln Glu Tyr Glu Lys Asn Pro Phe Asp Pro Val Ser Asn Pro Ser
        35                  40                  45

ggc atc atc cag atg ggc ctc gcc gag aac cag ctg tcg ttc gac ctg      192
Gly Ile Ile Gln Met Gly Leu Ala Glu Asn Gln Leu Ser Phe Asp Leu
    50                  55                  60

ctt gag gag tgg ctg gag aag aac ccc cac gcg ctc ggc ctc cgg cga      240
Leu Glu Glu Trp Leu Glu Lys Asn Pro His Ala Leu Gly Leu Arg Arg
65                  70                  75                  80

gag ggc ggc ggc gcc tcc gtc ttc cgc gag ctc gcg ctg ttc cag gac      288
Glu Gly Gly Gly Ala Ser Val Phe Arg Glu Leu Ala Leu Phe Gln Asp
                85                  90                  95

tac cac ggc ctc ccg gct ttc aaa aat gca ttg gcg cgg ttc atg tcg      336
Tyr His Gly Leu Pro Ala Phe Lys Asn Ala Leu Ala Arg Phe Met Ser
            100                 105                 110

gag cag aga ggg tac aag gtg gtg ttc gac ccc agc aac atc gtg ctc      384
Glu Gln Arg Gly Tyr Lys Val Val Phe Asp Pro Ser Asn Ile Val Leu
        115                 120                 125

acc gcc ggc gcc acc tcg gct aac gag gcg ctc atg ttc tgc ctc gcc      432
Thr Ala Gly Ala Thr Ser Ala Asn Glu Ala Leu Met Phe Cys Leu Ala
    130                 135                 140

gac cac ggc gac gcc ttc ctc atc ccc acc cca tac tac cca ggg ttc      480
Asp His Gly Asp Ala Phe Leu Ile Pro Thr Pro Tyr Tyr Pro Gly Phe
145                 150                 155                 160

gac cgc gac ctc aag tgg cgc acc ggc gcg gag atc gta ccc gtg cac      528
Asp Arg Asp Leu Lys Trp Arg Thr Gly Ala Glu Ile Val Pro Val His
                165                 170                 175

tgc gcg agc gcg aac ggg ttc cgg gtg acg cgc ccc gcg ctg gac gac      576
Cys Ala Ser Ala Asn Gly Phe Arg Val Thr Arg Pro Ala Leu Asp Asp
            180                 185                 190

gcg tac cgc cgc gcg cag aag cgc cgg ctg cgc gtc aag ggg gtg ctg      624
Ala Tyr Arg Arg Ala Gln Lys Arg Arg Leu Arg Val Lys Gly Val Leu
        195                 200                 205

atc acc aac ccg tcc aac ccg ctc ggc acc gcg tcg ccg cgc gcc gac      672
Ile Thr Asn Pro Ser Asn Pro Leu Gly Thr Ala Ser Pro Arg Ala Asp
    210                 215                 220

ctc gag acg atc gtc gac ttc gtc gcc gcc aag ggc atc cac ctc atc      720
Leu Glu Thr Ile Val Asp Phe Val Ala Ala Lys Gly Ile His Leu Ile
225                 230                 235                 240

agc gac gag atc tac gcc ggc acg gcg ttc gcc gag ccg ccc gcg ggc      768
Ser Asp Glu Ile Tyr Ala Gly Thr Ala Phe Ala Glu Pro Pro Ala Gly
                245                 250                 255

ttc gtc agc gcg ctc gag gtc gtg gcc ggg cgc gac ggc ggc ggc gct      816
Phe Val Ser Ala Leu Glu Val Val Ala Gly Arg Asp Gly Gly Gly Ala
            260                 265                 270

gac gtg tcc gac cgc gtg cac gtc gtg tac agc ctg tcc aag gac ctc      864
Asp Val Ser Asp Arg Val His Val Val Tyr Ser Leu Ser Lys Asp Leu
        275                 280                 285

ggc ctc ccg ggg ttc cgc gtc ggc gcc atc tac tcc gcc aac gcc gcc      912
```

-continued

```
Gly Leu Pro Gly Phe Arg Val Gly Ala Ile Tyr Ser Ala Asn Ala Ala
    290                 295                 300

gtc gtg tcc gcg gcg acc aag atg tcc agc ttc ggc ctc gtg tcg tcc      960
Val Val Ser Ala Ala Thr Lys Met Ser Ser Phe Gly Leu Val Ser Ser
305                 310                 315                 320

cag acg cag tac ctc ctc gcg gcg ctg ctc ggc gac agg gac ttc acc     1008
Gln Thr Gln Tyr Leu Leu Ala Ala Leu Leu Gly Asp Arg Asp Phe Thr
                325                 330                 335

cgg agc tac gtc gcg gag aac aag cgg cgg atc aag gag cgg cac gac     1056
Arg Ser Tyr Val Ala Glu Asn Lys Arg Arg Ile Lys Glu Arg His Asp
            340                 345                 350

cag ctc gtg gac ggg ctc agg gag atc ggc att ggg tgc ctg ccc agc     1104
Gln Leu Val Asp Gly Leu Arg Glu Ile Gly Ile Gly Cys Leu Pro Ser
        355                 360                 365

aac gcc ggc ctc ttc tgc tgg gtg gac atg agc cac ctg atg cgg agc     1152
Asn Ala Gly Leu Phe Cys Trp Val Asp Met Ser His Leu Met Arg Ser
    370                 375                 380

cgg tcg ttc gcc ggc gag atg gag ctc tgg aag aag gtg gtg ttc gag     1200
Arg Ser Phe Ala Gly Glu Met Glu Leu Trp Lys Lys Val Val Phe Glu
385                 390                 395                 400

gtc ggc ctc aac atc tcc ccc ggg tcg tcg tgc cac tgc cgc gag ccc     1248
Val Gly Leu Asn Ile Ser Pro Gly Ser Ser Cys His Cys Arg Glu Pro
                405                 410                 415

ggc tgg ttc cgc gtc tgc ttc gcc aac atg tcg gcc aag acc ctc gac     1296
Gly Trp Phe Arg Val Cys Phe Ala Asn Met Ser Ala Lys Thr Leu Asp
            420                 425                 430

gtc gcc atg cag cgc ctc agg tcg ttc gtc gac tcc gcc acc ggc ggc     1344
Val Ala Met Gln Arg Leu Arg Ser Phe Val Asp Ser Ala Thr Gly Gly
        435                 440                 445

ggc gac aac gcc gcc ctc cgc cgc gcc gcc gtc ccc gtc agg agc gtc     1392
Gly Asp Asn Ala Ala Leu Arg Arg Ala Ala Val Pro Val Arg Ser Val
    450                 455                 460

agc tgc ccg ctc gcc atc aag tgg gcg ctc cgc ctc acc ccg tcc atc     1440
Ser Cys Pro Leu Ala Ile Lys Trp Ala Leu Arg Leu Thr Pro Ser Ile
465                 470                 475                 480

gcc gac cgg aag gcc gag aga taa                                    1464
Ala Asp Arg Lys Ala Glu Arg
                485


<210> SEQ ID NO 13
<211> LENGTH: 487
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 13

Met Val Ser Gln Val Val Ala Glu Glu Lys Pro Gln Leu Leu Ser Lys
1               5                   10                  15

Lys Ala Gly Cys Asn Ser His Gly Gln Asp Ser Ser Tyr Phe Leu Gly
            20                  25                  30

Trp Gln Glu Tyr Glu Lys Asn Pro Phe Asp Pro Val Ser Asn Pro Ser
        35                  40                  45

Gly Ile Ile Gln Met Gly Leu Ala Glu Asn Gln Leu Ser Phe Asp Leu
    50                  55                  60

Leu Glu Glu Trp Leu Glu Lys Asn Pro His Ala Leu Gly Leu Arg Arg
65                  70                  75                  80

Glu Gly Gly Gly Ala Ser Val Phe Arg Glu Leu Ala Leu Phe Gln Asp
                85                  90                  95
```

-continued

```
Tyr His Gly Leu Pro Ala Phe Lys Asn Ala Leu Ala Arg Phe Met Ser
            100                 105                 110

Glu Gln Arg Gly Tyr Lys Val Val Phe Asp Pro Ser Asn Ile Val Leu
        115                 120                 125

Thr Ala Gly Ala Thr Ser Ala Asn Glu Ala Leu Met Phe Cys Leu Ala
    130                 135                 140

Asp His Gly Asp Ala Phe Leu Ile Pro Thr Pro Tyr Tyr Pro Gly Phe
145                 150                 155                 160

Asp Arg Asp Leu Lys Trp Arg Thr Gly Ala Glu Ile Val Pro Val His
                165                 170                 175

Cys Ala Ser Ala Asn Gly Phe Arg Val Thr Arg Pro Ala Leu Asp Asp
            180                 185                 190

Ala Tyr Arg Arg Ala Gln Lys Arg Arg Leu Arg Val Lys Gly Val Leu
        195                 200                 205

Ile Thr Asn Pro Ser Asn Pro Leu Gly Thr Ala Ser Pro Arg Ala Asp
    210                 215                 220

Leu Glu Thr Ile Val Asp Phe Val Ala Ala Lys Gly Ile His Leu Ile
225                 230                 235                 240

Ser Asp Glu Ile Tyr Ala Gly Thr Ala Phe Ala Glu Pro Pro Ala Gly
                245                 250                 255

Phe Val Ser Ala Leu Glu Val Val Ala Gly Arg Asp Gly Gly Gly Ala
            260                 265                 270

Asp Val Ser Asp Arg Val His Val Val Tyr Ser Leu Ser Lys Asp Leu
        275                 280                 285

Gly Leu Pro Gly Phe Arg Val Gly Ala Ile Tyr Ser Ala Asn Ala Ala
    290                 295                 300

Val Val Ser Ala Ala Thr Lys Met Ser Ser Phe Gly Leu Val Ser Ser
305                 310                 315                 320

Gln Thr Gln Tyr Leu Leu Ala Ala Leu Leu Gly Asp Arg Asp Phe Thr
                325                 330                 335

Arg Ser Tyr Val Ala Glu Asn Lys Arg Arg Ile Lys Glu Arg His Asp
            340                 345                 350

Gln Leu Val Asp Gly Leu Arg Glu Ile Gly Ile Gly Cys Leu Pro Ser
        355                 360                 365

Asn Ala Gly Leu Phe Cys Trp Val Asp Met Ser His Leu Met Arg Ser
    370                 375                 380

Arg Ser Phe Ala Gly Glu Met Glu Leu Trp Lys Lys Val Val Phe Glu
385                 390                 395                 400

Val Gly Leu Asn Ile Ser Pro Gly Ser Ser Cys His Cys Arg Glu Pro
                405                 410                 415

Gly Trp Phe Arg Val Cys Phe Ala Asn Met Ser Ala Lys Thr Leu Asp
            420                 425                 430

Val Ala Met Gln Arg Leu Arg Ser Phe Val Asp Ser Ala Thr Gly Gly
        435                 440                 445

Gly Asp Asn Ala Ala Leu Arg Arg Ala Ala Val Pro Val Arg Ser Val
    450                 455                 460

Ser Cys Pro Leu Ala Ile Lys Trp Ala Leu Arg Leu Thr Pro Ser Ile
465                 470                 475                 480

Ala Asp Arg Lys Ala Glu Arg
                485
```

What is claimed is:

1. An isolated nucleic acid comprising a promoter that functions in plants and further comprising a polynucleotide selected from the group consisting of SEQ ID NOS: 1, 2 and 4.

2. An isolated nucleic acid comprising a polynucleotide selected from the group consisting of SEQ ID NOS: 3, 5, 6 and 7.

3. The isolated nucleic acid of claim **1** comprising a promoter that functions in plants, wherein the polynucleotide comprises SEQ ID NO: 1 and SEQ ID NO: 2.

4. The isolated nucleic acid of claim **1** wherein said promoter is a constitutive promoter.

5. The isolated nucleic acid of claim **1**, wherein expression of the nucleic acid results in the downregulation of the expression of one or more endogenous ACS genes in a plant cell.

6. A plant or plant cell comprising the isolated nucleic acid of claim **1**.

7. A plant or plant cell comprising the isolated nucleic acid of claim **3**.

8. A plant or plant cell comprising an expression cassette effective for reducing expression of at least one endogenous ACS gene, wherein said expression cassette comprises a promoter that functions in plants operably linked to a nucleic acid configured for RNA silencing or interference, wherein said nucleic acid comprises a polynucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2.

9. The plant cell of claim **8**, wherein the plant cell is from a dicot or monocot.

10. The plant cell of claim **9**, wherein the dicot or monocot is maize, wheat, rice, sorghum, barley, oat, lawn grass, rye, soybean, *Brassica* or sunflower.

11. A plant regenerated from the plant cell of claim **10**.

12. The plant of claim **11**, wherein the plant exhibits one or more of the following: increased drought tolerance, increased nitrogen utilization efficiency, increased seed yield, increased biomass yield, increased density tolerance and increased density tolerance, compared to a control plant.

13. A method of reducing ethylene production in a plant, the method comprising reducing the expression of one or more ACC synthase genes in the plant by expressing a transgenic nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7.

14. The method of claim **13**, wherein the transformed plant exhibits one or more of the following: (a) a reduction in the production of at least one ACC synthase mRNA; (b) a reduction in the production of an ACC synthase; (c) a reduction in the production of ACC; (d) a reduction in the production of ethylene; (e) an increase in drought tolerance; (f) an increase in nitrogen utilization efficiency; (g) an increase in density tolerance; (h) an increase in plant height or (i) any combination of (a)-(h), compared to a control plant.

15. A method of increasing yield in a plant, the method comprising down regulating the expression of one or more ACC synthase genes in the plant by expressing a transgenic nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7.

16. A method of increasing drought tolerance in the absence of a yield penalty under non-drought conditions, the method comprising reducing endogenous ACS6 transcript levels or ACS6 activity.

17. An expression cassette consisting essentially of nucleotide sequences SEQ ID NO: 1 and SEQ ID NO: 2, wherein the nucleotide sequences are separated by an intervening polynucleotide.

18. The expression cassette of claim **17**, wherein the intervening polynucleotide is a ZmAdh1 intron 1.

19. The expression cassette of claim **18**, wherein the ZmAdh1 intron 1 sequence is bases 3791-4327 of SEQ ID NO: 3.

20. The plant of claim **8**, wherein endogenous ACS transcript levels or ACS activity is reduced relative to a control plant.

21. The plant of claim **20**, wherein the level or activity of ACC synthase is less than about 95% of that of the control plant.

22. The plant of claim **20**, wherein the level or activity of ACC synthase is less than about 85% of that of the control plant.

23. The plant of claim **20**, wherein the level or activity of ACC synthase is less than about 75% of that of the control plant.

24. The plant of claim **20**, wherein the level or activity of ACC synthase is less than about 50% of that of the control plant.

25. The plant of claim **8**, wherein the plant is maize, wheat, rice, sorghum, barley, oat, lawn grass, rye, soybean, sorghum, *Brassica* or sunflower

26. Seed of the plant of claim **8**, wherein the seed comprises the expression cassette.

* * * * *